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STUDIES OF CROWNGALL AND WOUND OVERGROWTH ON APPLE NURSERY STOCK[1]

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INTRODUCTION

As far back as records are available to the writers, it appears that a large percentage of apple nursery trees propagated by the root-grafting method have been affected by enlargements, or overgrowths, most of which ordinarily develop about the union of stock and cion. Much difference of opinion has existed both as to the cause of these enlargements and their effects upon the plants. Following the discovery by Smith and his coworkers (24, 25) of the causal relation of *Bacterium tumefaciens* Smith and Town. to galls on various plants, including the apple, the malformations so commonly found about the unions of root-grafted apple nursery trees have been rather generally attributed to the action of this organism, and the sale of crowngalled apple trees has been prohibited by law in many states. In their comprehensive accounts of crowngall, Smith *et al* (25, 26) report that *Bact. tumefaciens* occurs inside the cells of plants which they studied, stimulating abnormal activities which lead to the development of "primary tumors," "tumor strands," and "secondary tumors." While these papers contain no demonstration that "tumor strands" and "secondary tumors" occur in young apple trees, it has been widely assumed that such is the case, and that a tree once affected by crowngall is subject to the danger of recurring development of "secondary tumors." Recently, however, Riker (15, 16) in the United States and Robinson and Walkden (22) in England, working independently, have reported that, in the host plants which they studied, the crowngall organism was intercellular and that "tumor strands" appeared in their experiments only following infection in the region of a condensed bud where there was subsequent elongation of the internodes. Levine (7) has corroborated this work on "tumor strands." These contributions appear to show that crowngall infections are much more localized

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in their development than was previously supposed, and that there is no convincing evidence to demonstrate that "tumor strands" or "secondary tumors" play any significant part in crowngall of apply nursery stock. In consequence of these and other developments, a reconsideration of many aspects of the crowngall question seemed desirable. A committee representing the American Phytopathological Society, the American Society of Horticultural Science, the American Association of Economic Entomologists, and the American Association of Nurserymen was appointed to consider the question of official nursery inspection for crowngall. This committee held a crowngall symposium in connection with the annual meeting of the American Association for the Advancement of Science at Cincinnati in 1923 [see Stewart, (29)]. Among the points emphasized at this symposium were the difficulty of diagnosis of malformations on nursery apple trees and the inadequacy of available information concerning many important aspects of the general crowngall problem.

Following the Cincinnati meetings a research project was organized[2] whereby coordinated programs were initiated at the Iowa State College of Agriculture and Mechanic Arts and the University of Wisconsin. This paper contains an account of the work in progress at Wisconsin, preliminary reports of which have appeared (17, 18).

STUDIES OF CROWNGALL

A better understanding of the details of infection of grafted apple nursery stock by *Bact. tumefaciens* and of the characters of the resulting galls appeared to be of first importance. Accordingly, a study was made of the mode of infection by this organism and the development of the galls in various stages until their decomposition. For experimental plants the Wealthy variety of apple was chosen, because it is reported by many nurserymen to show commonly a very high percentage of root knot. Unless otherwise noted all the experimental apple trees and apple grafts mentioned in this paper are of the Wealthy variety. It was hoped that by adhering rather closely to one variety the results would be more nearly comparable. The plantings were all made, unless otherwise noted, in the greenhouses and gardens of the Department of Plant Pathology of the University of Wisconsin. Material both under glass and out of doors was given daily attention to provide water, drainage, sprays, and whatever was needed for favorable growing conditions.

[2] This work, which is being administered by the Crop Protection Institute through a committee consisting of I. E. Melhus (Chairman), G. W. Keitt, and M. F. Barrus, is supported cooperatively by the American Association of Nurserymen and individual nurserymen, the Iowa State College of Agriculture and Mechanic Arts, and the University of Wisconsin, in collaboration with the United States Department of Agriculture.

The crowngall organism appears to be capable of utilizing several different types of injuries to the external parts of the apple plant as infection courts. This was determined in an experiment in which injuries were made on the cion below the ground line on 20 one-year-old trees by a series of needle punctures, on 20 more by knife cuts, and on 20 others by bruises produced by blows from a hammer. In each case the crowngall organism used in previous work by Riker (15) was promptly applied in suspension in sterile distilled water to the surface of the wound. The technique was such, therefore, that the bacteria were not carried into the tissue in making the wound. The trees, which were dormant and rather dry at the time of injury, were planted May 1, 1925, and harvested the following fall. When the trees were dug those wounded by needle punctures showed eleven trees crowngalled, five smooth, and four dead; those wounded by knife cuts showed nine trees crowngalled, three smooth, and eight dead; while those wounded by hammer blows showed eleven trees crowngalled, four smooth, and five dead. A higher percentage of infection would probably have occurred if the organism had been carried into the tissue by the injury and if the trees had been actively growing instead of dormant at the time they were inoculated. Several of the dead trees bore the remains of decayed galls on the underground parts. From these experiments it is evident that crowngall infection may take place through any of the types of wound provided.

Infection of apple by *Bact. tumefaciens,* in conformity with reports by Smith *et al* (25) and Riker (15) for other plants, has been observed to occur only through wounds. The fresh unwounded callus on apple grafts appears not to be a common avenue of entry for this organism. Experiments on this subject were performed in the spring of 1924, when 100 grafts were prepared under conditions designed to exclude *Bact. tumefaciens.* The cions and stocks were dipped for one minute in an aqueous solution of mercuric cyanide, 1–1,000, and the knife was dipped before each cut in an aqueous solution of mercuric chloride, 1–1,000. After being callused in clean sand, the grafts were planted in soil on which no crop susceptible to crowngall had been grown for a number of years and which had just been steamed by the inverted pan method described by Johnson (4). As soon as the steaming was completed the treated soil was enclosed by boards to prevent washing by rain water. Successive lots of 10 of these grafts were inoculated at 10-day intervals, beginning with the day of planting. The earth was removed so as to expose the callus at the union, care being taken to avoid injury. The fresh callus and the earth immediately surrounding it were irrigated with one-half cc. of a concentrated water suspension of the crowngall organism which was used by Riker (15) in earlier studies. The soil was then replaced. In this experiment as in others the

fresh callus was found to be somewhat resistant to wetting. In cases in which the inoculum would not adhere to the surface it was placed on soil which was then brought in contact with the callus. Control inoculations by needle punctures on tomato and apple showed that the culture employed was pathogenic to both and that the environmental conditions of the experiment were favorable for the development of crowngall. When the trees were dug in the fall 85 were living but no crowngall was found. At the same time the above planting was made 60 commercial grafts which had been wrapped with unwaxed thread were dipped in a water suspension of the crowngall organism and planted in soil which had been liberally sprinkled five days previously with a similar suspension. Only one crowngall was found in this lot of trees when they were harvested at the end of the first growing season. Control inoculations by needle punctures on both apple and tomato produced well developed crowngalls. These experiments suggest that the callus at the union of apple grafts is not commonly an open infection court for the crowngall organism.

The above experiments were repeated in the growing season of 1925, when, however, only commercial grafts were planted. They were placed in soil which had not been steamed and which was not inoculated. As before 60 grafts were dipped in a water suspension of the crowngall organism at the time of planting. When they were dug in the following fall, none of them showed crowngall. As in the previous year 100 grafts were employed in an experiment in which the soil was removed from the unions of successive lots of 10 plants, at 10-day intervals, and one-half cc. of a water suspension of the crowngall organism was applied to the callus tissue and the soil at each union. When these plants were dug in the following fall only two of them had produced crowngalls. An additional series of 50 grafts was planted. On the day of planting three grafts received one-half cc. of the type of bacterial suspension just described. At three-day intervals, additional successive lots of three received similar treatments. When the grafts were dug in the fall none of them bore crowngalls. With each series of these experiments check inoculations on tomato and apple showed the cultures employed to be pathogenic to both and the environmental conditions favorable for the development of crowngall (Pl. XXXVIII, B). These results corroborate those of 1924 and indicate that, under the conditions of this experimentation, no part of the uninjured tissue of young apple trees, including the callus at the union of grafts, was commonly an open infection court for *Bact. tumefaciens*.

Though many writers on crowngall appear to have assumed that the callus tissue on apple grafts commonly serves as an open infection court for *Bact. tumefaciens*, the present writers have been able to find no record of convincing experimental evidence of such relationship. Until such evi-

dence is adduced, therefore, this assumption appears to be unwarranted.

Attention was next given to the characters of crowngall in various stages of its development on nursery apple trees. The crowngalls used for this work were induced by inoculations with the organism used in previous studies by Riker (15). The material studied comprised approximately 500 galls of different ages on grafts and young trees grown both in the greenhouse and in the field. The characters of these galls were indistinguishable from those of additional galls which occurred on apple trees from commercial nurseries and which yielded the crowngall organism in isolation studies. The term crowngall as employed in this paper refers to the true bacterial disease as it appeared in this material. It will be found that the description of crowngall which follows applies to only a small percentage of the knots found on commercial nursery apple trees. Other knots which have been confused with crowngall are described in a later section of this paper.

The microscopic appearance of crowngall on apple is quite similar to that described by Smith *et al* (26) for the Paris daisy and by Riker (15, 16) for the tomato. The interior of the gall proper appears to be composed for the most part of comparatively soft tissue interspersed with varying amounts of vascular elements. In some cases the gall, though comparatively soft in the early stages of its development, may have woody elements formed to such an extent that it becomes very hard (Pl. XXXIX, E, F, and Pl. XLII, A, B). The terms soft gall and hard gall may both be accurately applied to malformations induced on apple by the crowngall organism. However, the soft gall is the more common type. Regions of hyperplastic and hypertrophic tissue are readily found, but the more extreme types of the latter, as seen in the more succulent tissue of tomato and tobacco, did not appear in the apple material studied. In some places small centers of rapidly dividing cells may be seen which appear to be initiating new regions of hyperplasia. Cells with walls which are thickened in the manner of xylem tissue sometimes appear as isolated units and at other times they seem to approach the organization found in vascular bundles. In places, in connection with these woody elements, the arrangement of certain cells has suggested medullary rays, cambium, and cortical elements. In some of the galls structures were observed which gave the appearance, within a limited area, of several layers of cambium arranged at different distances from the surface (Pl. XLII, A, b, b′). The gall appears to enlarge by growth within the softer tissues beneath the surface, rather than by the callus type of growth (Pl. XLII, D, E), as described on a later page, or by the type of secondary thickening that follows cambial activity. The structure of the gall varies considerably at different stages of development and in different parts. It is common to observe tissue in the gall which is quite similar to callus and wound tissue. It has already

been suggested that a very close relationship exists between the pathological developments stimulated by the crowngall organism and those by various chemicals (27) and injuries (20). The surface of the gall appears not to consist of any specially differentiated tissue. It is quite common to find the outer cells dead (Pl. XLII, A, a).

The gross appearance of crowngalls (Pl. XXXVIII, A, B, C) is in close conformity with the characters observed under the microscope. The surface, which is comparatively smooth like that of crowngalls on peach and raspberry, is frequently covered by dead cells. A definitely organized cork layer has not been found on the gall proper but it may occur in connection with wound tissue wherever the crowngall is associated with wound overgrowth. However, the dead cells on the surface sometimes resemble cork. The granular, frosty appearance of the surface of fresh callus is not common and when it does occur it is usually limited to rather small areas of the surface of older galls. On the larger galls it is frequently found that certain portions have begun to decay. Small roots or root primordia seldom if ever originate from the crowngall proper. In cases in which roots have been observed in association with a crowngall they have been found to occur in connection with wound overgrowth that was adjacent to the gall. Wound tissue has been found in such position on a small percentage of the trees as if in response to an injury produced by the tumor. This is in conformity with a report by Muncie (11) that an infectious type of hairy root may arise from a gall. The gall tissue, as Smith *et al* (25) have reported, contains more oxidase than cortical tissue and turns brown more quickly upon exposure to the air. Crowngalls on the above ground portions of apple trees have been found to be smaller and harder than those below the soil line. This is probably due largely to the drying influence of the air as shown by Riker (19) on raspberry.

The characters of crowngall are sometimes obscure, especially on material collected in the nursery, because crowngall proper and excess callus or wound overgrowth, which are described later, may merge in a common enlargement. Crowngall may also occur from infection of callus or wound overgrowth. Since all of these overgrowths are initiated in wounds and are somewhat similar in gross characters, it is not surprising that they have been confused.

The identity of the crowngalls employed in these studies was checked by demonstrating through numerous isolation and reinoculation tests that they contained the crowngall organism. *Bact. tumefaciens* has been found to be continually present in undecayed portions of crowngalls at all stages of their development. Some of the galls studied were of considerable size and were doubtless several years old. The details of isolation technique and the number of enlargements examined are given on later pages in connection with investigations of wound overgrowths.

Difference of opinion exists with regard to the seat of the stimulus which incites the development of crowngall. The following facts suggest that the bacteria within the gall, rather than those on the surface as reported by Robinson and Walkden (22) for daisy, furnish the stimulus causing the development of crowngall on apple: (a) *Bact. tumefaciens* was isolated consistently and in abundance from the interior tissues of known crowngalls, (b) growth of crowngalls occurs primarily from the interior rather than the surface cells, and (c) the presence of the causal organism on the unbroken surface of host parts, even on fresh callus, did not induce gall development in the experiments reported above.

Galls induced by the crowngall organism appear under certain conditions to be subject to decay by secondary invaders. As reported above, a considerable number of the galls resulting from inoculations on grafts and one-year-old trees showed various stages of decay when they were dug in the fall. This fact is not surprising, since crowngall tissue is commonly soft and unprotected. It has been frequently observed in storage that, under conditions of comparatively high temperature and moisture in the spring, the rot organisms attacked the cortex of crowngalled trees in such manner as to girdle them. This type of injury has been observed to occur also on crowngalled trees in the field. Injury of this character was responsible for the loss of many of the inoculated grafts and trees in the greenhouse in two separate Wisconsin temperature tank (Jones, 5) series set up to determine the influence of soil temperature and soil moisture on the development of crowngall on apple. Practically all the apple trees at temperatures above 20° C. and at the same time in soil with a high moisture content were killed in this way. On the other hand, crowngalls have been kept very well in storage under conditions of low temperature and low moisture. Crowngalls from the previous year have been found in excellent condition on both apple and raspberry in the field in Kansas during the month of March and in Wisconsin during May. In some cases where the gall was badly decayed new gall tissue has been found to grow from the margin. Cultures of the crowngall organism have been secured without difficulty from these several types of specimens. So it is evident that, although crowngall on apple is quite subject to decay, it can persist from one growing season to the next on the host plant and thus supply a new potential source of inoculum after the winter.

Injury incident to crowngall infection of young apple trees has been observed to lead to unsatisfactory growth or death. The constriction-like effect described by Levine (6) and the reduction in water flow shown by Muncie (12) are probably factors in the inhibition of growth. However, the most serious source of injury probably results from the rot induced by secondary invaders. It appears that sooner or later bacterial crowngalls are

largely destroyed by such invaders, which are likely also to attack the cortex of young trees and induce girdling.

Various antiseptics were tried in the early phases of this work in an effort to control crowngall on apple. Among those employed were Bordeaux mixture, copper carbonate, lime-sulphur, sulphur, dry lime-sulphur, slaked lime, bleaching powder, lithium carbonate, sodium silicate, zinc chloride, ammonium hydroxide, potassium permanganate, ferric chloride, ferric sulphate, aluminum chloride, tannic acid, furfural, toluol, carbon bisulphide, laundry soap, and several organic mercurial compounds. Before they were tried on apple grafts in the field an idea of their toxicity to plants and their efficiency against crowngall infection was gained by tests on tomato plants in the greenhouse. These experiments indicated that with the materials used treatment previous to inoculation was of comparatively little value, probably in part because the wounds which were made to permit the entrance of the bacteria broke through the covering provided by the antiseptics. Treatment subsequent to inoculation was effective only when applied within a short time and then only where the antiseptic killed the cells that formed the infection court. About 11,000 grafts were treated in the field in lots varying from 60 to 200. In some cases the cions and roots were treated before the grafts were made. The antiseptics were applied only to grafts which were wrapped with waxed thread. The grafts were treated by dipping them into solutions, by dusting them, by planting them in sprayed and also in dusted trenches, and by puddling them in soil which had been treated with various antiseptics. These experiments were conducted for the most part at the North Star Nursery at Pardeeville, Wisconsin. The details of this long series of experiments are omitted because no satisfactory control of the malformations dealt with was secured.

A commercial preparation of hydroxymercurichlorophenol gave less promise of control of crowngall than some of the other compounds when tried against experimental inoculations. This was tested first on tomato plants. Twelve tomato stems were punctured five times each with a sterile needle. The first three plants were dipped in a solution of the commercial product, 1–400, and after an interval of a half-hour received a water suspension of the crowngall organism. In all the inoculations of this series the bacteria were applied with a camel's hair brush. The stems were still wet with the hydroxymercurichlorophenol solution at the time the bacteria were applied. The next three plants received the same treatment except that the bacteria were applied immediately after the plants were dipped in the solution and while the stems were well covered with it. The next three tomato plants received the application of the bacterial suspension promptly after wounding and were then dipped in the solution after an interval of less than one minute. The last three plants received the same treatment

except that a half-hour elapsed between the application of the bacteria and the treatment with the solution. The plants were all placed in the greenhouse under favorable conditions for the development of crowngall. The chemical produced no visibly injurious effect upon either the tomato stems or leaves. After three weeks the 12 tomato plants each showed five well developed crowngalls. This experiment casts doubt upon the value of hydroxymercurichlorophenol as a direct preventive of crowngall infection. A similar experiment was tried with another commercial preparation of hydroxymercurichlorophenol manufactured by a different company. Like results were obtained.

When apple grafts were dipped in a solution of hydroxymercurichlorophenol, 1–400, it was noticed that the fresh young callus became somewhat brown. This discoloration appeared to a less extent following dipping in water, and in a still smaller degree after exposure to the open air. The injury from several mercurial preparations was considerably more conspicuous when they were employed in the form of a dust. Many other chemicals used produced considerable injury to the fresh callus.

The possibility of controlling infection of grafted apple nursery stock by the crowngall organism with antiseptics appeared still less promising when it was learned that the fresh callus, the part which it was sought particularly to protect, probably is not ordinarily an open infection court for *Bact. tumefaciens*. Furthermore, as the experimental work progressed, it became evident that the great majority of the malformations encountered below the soil line on grafted apple trees in the nursery, aside from the well known enlargements caused by woolly aphis and nematodes, were not caused by *Bact. tumefaciens*. Consequently, it became necessary to shift the major attention from crowngall to these other malformations. Several recent reports of the control of "crowngall" by antiseptics are discussed on later pages.

STUDIES OF CALLUS AND WOUND OVERGROWTH

Since the malformations found on most of the root-grafted apple trees rejected as crowngalled at the nurseries had different characters from galls produced on young apple trees by inoculations with cultures of *Bact. tumefaciens*, an attempt was made to determine their nature, cause and control.

Superficially, these enlargements resembled the bacterial galls. However, closer examination in their various stages of development revealed distinct differences. The first stages observed were callus developments about wounds. In the early stages, growth of this callus occurred chiefly through the division and enlargement of the surface cells (Pl. XLII, E; XLIV, B; and Fig. 1). This tissue, which had a white, frosty appearance when fresh, was somewhat resistant to wetting but very susceptible to injury from

various chemicals, or even from exposure to the open air. On later pages these early stages of development are referred to as callus. While such callus tissue was occasionally found on parts of overgrowths which were as much as three years old, more commonly a phellogen was differentiated in the first or second year and a cork layer formed. From this time growth of the knots appeared to result chiefly from the activity of a cambium layer. Their interior was commonly, but not always, very hard, due to the presence of wood. Indeed, it was often more difficult to cut with a knife than the stem proper because of the irregularity in arrangement of the woody elements. This interior tissue appeared to contain much less oxidase than that of crowngall (Pl. XXXIX, E). In the succeeding pages, in view of evidence which will be presented, these malformations in this stage are called wound overgrowths. These enlargements were often very irregular in contour, due partly to the development of root primordia and roots. Zimmerman (34) has reported similar developments on hard-wood cuttings. Not infrequently a considerable number of roots grew out and produced the appearance of the common "woolly knot" (Pl. XXXIX, D). In some cases very

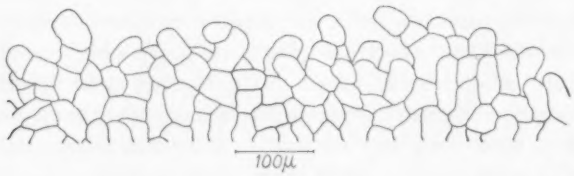

FIG. 1.—Section at the surface of fresh young callus on a Wealthy apple graft. The cells at the surface were alive and actively dividing. Drawing made with the aid of a camera lucida.

peculiar forms were produced by abortive and fasciated root development, which had some resemblance to balls of knotted string. Sometimes, as in Plate XXXIX, A, and XL, C, D, the diameter of the main axis below the enlargement was less than that above. As has been mentioned before, cases occasionally were found in which wound overgrowths occurred in combination with crowngall.

In view of the fact that the malformations just described have commonly been supposed to be crowngall, a bacteriological study of these enlargements was initiated. A standard technique was adopted with the aim of securing the maximal feasible accuracy and consistency from the method in the hands of several operators. The writers were very ably assisted in these isolation studies by Paul Miller, Ismé Hoggan, L. W. Boyle, and W. M. Banfield. Each of the individuals who performed this isolation work practiced on known crowngall material until he was able to isolate the causal organism consistently. Only after the technique was well mastered was he permitted to work on this project.

The procedure in the isolation work during the winter of 1924–1925 was as follows. The culture medium employed was composed of 17 gm. of agar, three gm. of beef extract, 10 gm. of peptone, 10 gm. of dextrose and 1,000 cc. of distilled water. The medium was prepared in the usual manner and adjusted to pH 7.0. The surface of the malformation to be examined was thoroughly cleansed with water. A portion of the interior was exposed with knives which were dipped in alcohol, flamed, and manipulated in accordance with the usual technique employed for securing interior tissues free from external contamination. By proceeding in this manner a portion of tissue was secured which was nearly or entirely free from surface contaminations. In the early phases of the work the surface of the gall was treated with mercuric chloride, 1–1,000, but this practice was soon discontinued because it appeared to reduce the chances of securing the crowngall organism. After a small bit of tissue, approximately one-sixteenth to one-eighth inch cube, was exposed under the conditions just described, it was removed to one cc. of sterile distilled water in a sterile petri dish where it was crushed or cut into very small portions. By this procedure fragments were removed from at least five different representatively distributed parts of the knot under examination including outer and inner portions, a separate petri dish being used for each portion of tissue cultured. After the crushed tissue had stood in the sterile distilled water approximately one-half hour to permit the diffusion of bacteria the plates were poured in the usual manner. At one time nutrient broth instead of distilled water was employed, but under the conditions of this work there appeared to be no advantage in using broth. The plates, which were incubated at approximately 22° C., were examined at frequent intervals and puncture inoculations into tomato stems were made from colonies which most resembled those of *Bact. tumefaciens*. Similar inoculations were also made from colonies of various types. For possible future study, transfers to agar slants were made from many of the colonies chosen for inoculation. These colonies were usually selected on the fourth or fifth day. However, no plates were discarded without being examined after they were at least 10 days old. Check inoculations with a known culture of the crowngall organism were made with each series. All the inoculated and control plants were kept, appropriately segregated, on a greenhouse bench where the temperature was approximately 22° C. and other conditions were favorable for the development of galls. Although the tomato plant was always employed, inoculations were also made from time to time on tobacco and geranium. With the various cultures used, these plants consistently yielded results which conformed with those on tomato. Ordinarily under the conditions of these studies the galls developed from inoculations with *Bact. tumefaciens* on tomato so as to be visible in seven days and conspicuous in ten days. However, the results

were never recorded as negative until an examination was made at least a month after the inoculation. The results on herbaceous plants were checked by inoculations on apple. Twenty-two inoculations on apple were incubated for two months, and 50 more on apple were incubated for an entire growing season. At the end of these periods all the trees were free from any enlargement, except those inoculated with the cultures which were positive on tomato. In every case the original knot cultured was kept and numbered in case further examination might be desired. Records which included entries under forty-nine different heads were kept for each knot examined. A sample of the record sheet is omitted because of its size. The writers understand that this technique is similar to that employed by Brown (1) in her work on the apple stem tumor. All cultural examinations referred to on later pages were made by this technique or the slightly modified method used in 1925–1926.

The efficiency of the isolation technique was tested at frequent intervals. For approximately every five trees cultured a check isolation was performed on crowngall of apple induced by inoculation or on crowngall of peach, cherry, or raspberry from natural infection. In 1924–1925 positive results were obtained from 44 of 46 such crowngalls. In this work, it was very common to secure the crowngall organism from three or four of the five pieces of gall selected for plating. In general practice it was found that on the average the crowngall bacteria were secured from approximately seven of every ten of individual pieces tried from known crowngall material. Consequently five separate attempts were made from each knot because it was desired to maintain a high degree of accuracy in the technique employed. It is considered very important that numerous checks should be made on the technique of isolations, because otherwise certain errors might creep in and reduce the accuracy of the studies.

Attention is invited to several other points in the procedure at which error may enter unless due precautions are taken. (a) It appears to be necessary for success in making isolations that the material should be fresh and the portion used should be free from decay. Two dozen attempts at isolation from decayed crowngall tissue have given uniformly negative results. A consideration of what becomes of the crowngall organism in decayed tissue is beyond the scope of this paper. (b) When inoculations are made on tomato plants to test the pathogenicity of the different cultures precautions should be taken to prevent the bacteria from being spread from one inoculation to another when the plants are watered. The possibility of this means of transfer was tested by placing a half dozen potted tomato plants inoculated in 52 places among a half dozen plants which were wounded by needle punctures in 230 places but uninoculated. All the plants, which were placed side by side on a greenhouse bench, were then watered with a

hose in the usual manner. After three weeks infection was found at 92 per cent of the punctures on the inoculated plants and at 21 per cent of the punctures on the wounded but uninoculated plants. (c) Another possible source of error is in the manner of making injuries for inoculations. This is especially important with woody plants, as apple, but is also important with some of the more herbaceous plants. The resemblance between crowngall and wound reaction on tomato has been discussed by Riker (20). In cases in which large wounds were made in woody plants, but no inoculum applied, small rather hard enlargements were sometimes produced after a considerable period. These resulted from the wounding, without the intervention of *Bact. tumefaciens*. Such overgrowths, however, might be confused with crowngall if not understood. These experiments are described on later pages. Meanwhile, it should be observed that infection with *Bact. tumefaciens* may be obtained consistently without making large wounds. (d) Certain other types of reaction which appeared to be associated with bacteria, but which, however, are not typical of crowngall, deserve more detailed consideration.

The interiors of the wound overgrowths on the apple material cultured were found sometimes to be sterile and sometimes to contain various types of bacteria. In agar plates some of these organisms produced colonies that resembled those of *Bact. tumefaciens*. The presence or absence of the different bacteria varied with the organization of the woody and softer elements. In places in which the usual type of cortex, as it occurs in apple stems or roots, covered a regular formation of xylem elements, bacteria very rarely were found. However, they often were isolated from places in which the wood and soft tissue elements were very considerably gnarled and twisted (Pl. XL, B, D and Pl. XLII, C). In such locations it was not uncommon to find small particles of soil which had probably been incorporated in the tissue as it enlarged in close contact with the soil. Different organisms from such regions showed a wide range of growth characters, fermentation reactions on sugar media, and dye absorption. The details of these experiments are omitted from this paper because the studies are incomplete. When the bacteria found in the wound overgrowths were inoculated into tomato the wounds in most cases corked off in the same manner as wounds made by puncture with a sterile needle. Such wound response in tomato has been observed to be comparatively small except at times in soft tissue near the top of the stem. In this position it has sometimes produced an enlargement which attained an elevation of one or two mm. in a month. A few of the bacteria isolated from wound overgrowths, however, appeared to induce certain reactions, three of which deserve mention. (A) In certain instances various degrees of rotting of the tomato stem tissue about the point of inoculation have resulted. The more extreme types suggested the

rot induced by *Bacillus carotovorus* Jones on carrot. Such a reaction has not been observed to be induced by *Bact. tumefaciens.* (B) In some cases the type of development just mentioned was followed several weeks after the inoculation by a slight enlargement. This was ordinarily quite small in comparison with crowngalls of the same age. (C) In some other cases rather similar swellings in the tomato stem tissue at the point of puncture were noticed where no rotting of the tissue occurred. These were very small in comparison with crowngalls of the same age which were induced by a known culture, being only slightly larger than the swelling which sometimes follows the puncture with a sterile needle. Similar swellings have been noted occasionally to develop about wounds in which either steamed or unsteamed soil had been placed. They suggest some of the swellings which were induced by Smith (27) with various chemicals. The identity of the organisms that appear to be associated with these enlargements has not yet been determined. In view of the facts that (a) they were secured from knots on apple which did not resemble those induced by known cultures of the crowngall organism, (b) in some cases their cultural characters were different from those of this parasite, and (c) the reaction which they induced on tomato stems was different from that induced by this organism, the question is raised as to whether or not they should be called *Bact. tumefaciens.* It appears that considerably more evidence of every kind will be necessary before these organisms can be identified. They may be either strains of the crowngall organism or distinct from it. They have been found in less than ten per cent of the knots examined. The organisms which were isolated from the interior of wound overgrowths, but which induced on the tomato stems no swelling larger than wound reactions to the inoculating needle, have not been identified. When placed in needle punctures they have failed to induce any suggestion of crowngall on tomato, geranium, tobacco, or apple. It is not surprising that the colonies of some of these bacteria on agar plates resembled those of the crowngall organism, since the distinguishing characters of bacterial colonies on agar plates are comparatively few and there are many kinds of bacteria in the soil. It seems probable that these bacteria were soil organisms which gained entrance to the tissues of the wound overgrowths and survived.

Smith *et al* (25) report differences in cultural characters of certain strains of *Bact. tumefaciens.* Limited studies of this subject by the present writers have yielded similar results.

For the isolation studies of 1924–1925 approximately 1,500 root-grafted apple trees which were representative of those rejected as crowngalled were secured from twelve of the leading nurseries in the states of Wisconsin, Iowa, Nebraska, Kansas, Missouri, and Tennessee. To these were added approximately 500 trees from plantings at Madison, Wisconsin. By means

of the technique described above, an examination was made of the overgrowths of 227 of these trees which were selected at random to include representative specimens from each lot. The trees were of various sizes and ages. Of the total number 103 were less than one year old, having been collected at intervals from the day of planting to the end of the growing season. Of the 227 malformations approximately two per cent had the characters of crowngall as described above. These two per cent readily yielded bacteria which, when inoculated into tomato, geranium, tobacco, or apple induced crowngalls indistinguishable from those caused by known cultures of the crowngall organism. Approximately eight per cent more of the malformations yielded organisms which, when inoculated into susceptible plants, induced slight swellings which were much smaller and slower to develop than those induced by *Bact. tumefaciens*. These eight per cent possessed either the characters of wound overgrowths or a mixture of those of wound overgrowths and crowngall. The relationship of these bacteria to the enlargements from which they came has not been determined. The other 90 per cent of the enlargements examined had the characters of wound overgrowths. The interiors of these enlargements were either sterile or yielded organisms that did not produce any evident reaction upon tomato, geranium, tobacco, or apple. The evidence from these studies strongly suggests that the crowngall organism is responsible for only a comparatively small portion of the malformations on the nursery apple trees studied.

In the season 1925–1926 the results obtained were similar to those of the previous year, except that a slightly higher percentage of crowngall was found. The material cultured was obtained from 15 nurseries in the states of Arkansas, Illinois, Iowa, Kansas, Michigan, Minnesota, Missouri, New York, Pennsylvania, and Wisconsin. Most of these nurseries were different from those which supplied the trees used in the season 1924–1925. The isolation technique was varied from that used the year before by employing a medium similar to that used by Wright (33). A 10 per cent stock solution of yeast water was prepared by placing 500 grams of starch-free yeast in 5,000 cubic centimeters of water, steaming for two hours, and autoclaving for one hour at 15 pounds pressure. After the insoluble material had settled for several days the clear supernatant liquid was used. The medium was made of the following components:

Stock solution yeast water	100.0 cc.
Distilled water	900.0 cc.
Di-basic potassium phosphate (K_2HPO_4)	4.0 gm.
Sodium chloride (NaCl)	.2 gm.
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	.2 gm.
Calcium carbonate ($CaCO_3$)	3.0 gm.
Dextrose ($C_6H_{12}O_6$)	10.0 gm.
Agar	20.0 gm.
Reaction adjusted to pH 6.8	

Examination was made of malformations on 156 trees. These were taken at random except that no enlargements were included which were comparatively small in relation to the diameter of the tree. Eleven per cent were positive for crowngall, 8 per cent were doubtful as described earlier, and 81 per cent were negative. In addition to these specimens, 24 more were selected for culture because they showed characters of both wound overgrowth and crowngall. Of this number 13 per cent were positive for crowngall, 25 per cent were doubtful, as described earlier, and 62 per cent were negative. Of the 21 knots which were positive for the crowngall organism, four showed a predominance of the characters of wound overgrowth, five showed a mixture of those of wound overgrowth and of crowngall, and 12 showed only the characters of crowngall. Some mixture of the characters of crowngall and wound overgrowth is to be expected, since both types of malformation depend upon injuries for their initiation. As in the preceding year, the efficiency of the technique was tested against known material at frequent intervals. Positive results were obtained from 26 or 27 known crowngalls on inoculated apple trees and naturally infected peach, plum, and cherry. The difference in the percentage of crowngall on the trees examined in 1924–1925 and in 1925–1926 is not surprising when consideration is given to the facts (a) that the material studied in the two seasons came for the most part from different locations, (b) that variation from season to season in the percentages of both wound overgrowth and crowngall is to be expected, and (c) that the small malformations were not included in the studies made in 1925–1926.

A review of the results of the isolation studies in the two seasons show that the technique used gave positive results from 70 of the 73 control trials with known crowngall, and negative results from a high percentage of the enlargements which possessed the typical characters of wound overgrowths. Although much care has been exercised to develop and check the accuracy of the isolation technique, the possibility which is inherent in the negative aspect of the results still remains that further study may possibly reveal the presence of a causal organism in wound overgrowths. However, in view of the facts (a) that Hedgcock (3) reports no isolation studies, (b) that Smith *et al* (25) report only a few isolations from apple which may or may not have been from the type of malformation under consideration and which yielded a considerable percentage of negative results (25, p. 53), (c) that the cultural studies reported herein have consistently failed to reveal the crowngall organism in typical wound overgrowths, although the same technique isolated it consistently from known crowngalls, and (d) that wound overgrowths have been induced or suppressed practically at will under conditions designed to exclude the crowngall organism, as reported on later pages, there seems to be no justification at present for concluding that *Bact. tumefaciens* is the cause of these malformations.

The juncture between the cion and stock appears to be intimately associated with the development of most of the wound overgrowths. An examination of the surface characters of the malformations on two- and three-year-old trees which were grown from piece-root grafts showed that approximately 85 per cent occurred at the union. Of this number about 90 per cent were associated with the lower tip of the cion. This relationship is more clearly observed when sections are made through the trees in such manner as to reveal the original union. Surface and sectional views of such trees may be seen in Plates XXXIX, A, B, C, and XL. In many cases the original wood of both the cion and stock may be observed. Such cases reveal very clearly the tendency for the enlargements to develop about the lower tip of the cion at a place where cambial connection with the root either has been quite poor or has failed entirely. The origin of these knots on the lower cion tip is more easily observed in one-year-old trees. Sectional and surface views of some of these may be seen in Plates XLI and XLVI, B, C, D. Here again, but more clearly than before, it appears that these enlargements have their origin in the lower end of the cion which has not been fitted properly to the root. Sections through one-year-old trees often reveal the original wood of the graft and show how the grafted parts were fitted together. It may be observed in Plate XLI, A, C that the cambium on the lower tip of the cion was not matched against that of the root in such manner that union of these parts could take place.

Callused grafts provide an excellent opportunity to observe the initiation of these enlargements. Such grafts may be seen in Plate XLIV, B. Here it appears that the beginning of knots may follow certain types of misfit in grafting. It is conspicuous that grafts which have the lower cion tip cut and placed in such a manner that the cambium layers of the cion and stock do not closely approach each other are quite likely to develop callus overgrowths. Such a condition is particularly apt to occur when the lower cion tip is too long (Pl. XLIII, C) or when the cion is larger in diameter than the root (Pl. XLIII, F). It may also occur if the lower tip is blunt (Pl. XLIII, D), if it is cut in such a manner that it naturally stands away from the stock when the graft is not tightly wrapped, or if the wrapper rots away too quickly (Pl. XLIII, B). It appears to be important that the wrapper should hold tightly until union between the cion and root becomes well established. Otherwise there seems to be a tendency for a cushion of callus to form under the lower cion tip and to push it away from the stock (Pls. XLI, A to C and XLIV, A, B). In some cases this appears to be serious enough to initiate the development of a knot. Cases in which the cambium of the lower cion tip is cut short of that on the root (Pl. XLIII, A) or in which it projects over very slightly are of less importance to the initiation of wound overgrowths.

Thus, it appears that the development of most of the wound overgrowths may be traced to a beginning in poorly united cions and stocks. Since no causal organism has been found to be associated with the great majority of these enlargements in any stage of their development, it seems necessary to seek other causal factors. The consequences of wounds appear to be, in themselves, sufficient to lead to the production of these overgrowths. A discussion of some of the physiological aspects of the development of wound overgrowths is given on a later page. Meanwhile, a consideration of the fit of a number of commercial grafts is pertinent.

A variety of misfits at the union was found in different samples of commercial apple grafts which were examined. Since the fit at the lower tip of the cion appeared to be particularly important the grafts were classified according to the position of the cambium on this part of the cion relative to that on the stock. Several different classes were arbitrarily chosen. The class "short" (Pl. XLIII, A) included the grafts in which the cambium of the lower cion tip did not come down as far as the cambium on the root. Class "even" (Pl. XLIII, B) included those in which the cambium layers were in close proximity at this critical point. Class "over" included those in which the cambium of the cion tip extended below that of the root by less than 1/16 inch. Class "long" (Pl. XLIII, C) included those in which the cambium of the cion tip extended below that of the root by more than 1/16 inch. Class "blunt" (Pl. XLIII, D) included those in which the lower cion tip had been long, but was cut off at the time the graft was made. In addition to these types a considerable number of grafts were found on which the cambium layers at the lower cion tip were opposite each other, but which were wrapped in such manner that these cambial surfaces were not brought in contact. Such grafts have been included under the additional classification "loose wrap" (Pl. XLIII, B). In grafts where the diameter of the cion was greater than that of the stock, a certain portion of the cambium of the cion obviously could not come in contact with that of the root. Such grafts were given the additional classification "cion large" (Pl. XLIII, F). The results of classifying ten samples of grafts into these different groups may be seen in table 1. Sample No. 1 was received with the notation "well fitted grafts." This was true as applied to the relative diameters of cions and stocks, but not with reference to the fit at the lower cion tip. In view of this notation the question is raised as to whether earlier investigators (Hedgcock, 3, and Melhus and Maney, 8) found comparatively little difference between well fitted and poorly fitted grafts in relation to the development of enlargements because their criterion of fit may have been based more on relative diameters of the stock and cion, and less on the matching of cambium layers at the lower cion tip. From an examination of this table it appears that a sufficient number of misfits is present among the grafts to

TABLE 1.—*Classification of ten samples of commercial apple grafts according to the fit of cion to stock*[a]

Sample number	Total no. examined	Short	Even	Over	Long	Blunt	Loose	Cion large
		Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
1	520	20	43	19	17	1	16	1
2	100	8	29	23	14	26	79	4
3	417	12	14	4	16	54	62	15
4	4403	15	15	16	27	27	33	1
5	860	16	30	16	22	16	46	0
6	1041	2	13	5	21	59	0	0
7[b]	84	36	47	17	0	0	0	0
8	134	31	30	20	17	2	0	0
9	205	9	49	21	17	4	0	0
10	214	7	3	14	66	10	0	0
Average		16	27	15	22	20	23.6	2.1

[a] For explanation of classifications used see text.

[b] This sample was sent in as "perfectly fitted grafts."

account for most of the wound overgrowths developed in the field. This subject will be discussed more fully on later pages.

The number of poorly fitted grafts is not surprising when one considers the speed with which they are prepared. In some places the workmen who make the grafts are required to cut and fit at least 3,000 in a ten-hour day, an average of one in 12 seconds. For each graft a slanting cut and a cut for the tongue must be made at the lower end of the cion, and a cross cut at the top. Three similar cuts must be made on the root. After this the cion and a root of the proper diameter must be fitted together. Under these conditions a considerable number of misfits would seem to be inevitable.

Although the lower tip of the cion is the most common location at which wound overgrowths occur, they are sometimes found elsewhere. They may be found extending along the side of the union (Pl. XLVII, D). This is especially apt to occur when the cion is larger than the stock (Pl. XLIII, F) and consequently a considerable portion of the cambium on the cion is unable to make contact with that on the root. It occasionally happens, too, that a knot will be formed at the point of the tongue which extends down from the cion. At times this portion of the cion extends out from the root in such manner that the cambium does not make contact (Pl. XLIII, F, b). Knots have also been found just above the upper tip of the root (Pl. XLVI, B, b). Wound overgrowths seem more apt to occur in this position when a very long slant has been made on the cion and a portion of the cut surface is not covered by the root. In addition to these malformations which appear

to be associated with poor fit of the grafted parts, knots were sometimes found on unions which were first smooth, but later wounded.

Mechanical injuries often appear to initiate the knots which develop on the cion wood of the young apple trees above the unions. Such injuries are commonly made by the careless use of cultivators, hoes, and other tools and by insects. Where wounds remove a portion of the bark and expose the woody part of the stem the results closely parallel those which follow poor fits in grafting. Under favorable environmental conditions callus is formed in a short time and later wound overgrowth develops (Pl. XXXIX, A, B, D). Five to ten per cent of the wound overgrowths on grafted apple trees and nearly all those on budded trees appeared to be initiated by mechanical injuries. During the time a tree stands in the nursery row there is considerable opportunity for injury of underground parts. In the specimen shown in Plate XXXIX, A, B, the diameter of the main axis above the wound overgrowth is considerably greater than that below. This condition suggests that a considerable portion of descending food passed into the roots which originated in the wound overgrowth. Such a situation is not uncommon in wound overgrowths, but has not been observed in cases of known crowngall. Although apparently not so important as the proper fitting of grafted parts, mechanical injuries are a noteworthy factor in the production of wound overgrowths.

Thus far evidence has been presented to show that wound overgrowths are much more numerous on apple trees in the commercial nurseries than crowngall, that they differ from crowngall in various characters, that they appear not to be induced by the crowngall organism, that they develop following poor fits in grafting and mechanical injuries, and that in commercial propagation a sufficient number of poorly made grafts is ordinarily found to account for most of the wound overgrowths which develop in the nursery. A consideration of the experimental production of callus and wound overgrowths follows.

PRODUCTION AND SUPPRESSION OF CALLUS AND WOUND OVERGROWTH

A brief consideration of the development of excess callus and wound overgrowth may be of assistance in the interpretation of results of experiments which deal with their production or suppression. It is commonly accepted that the elaborated foods which are produced by the leaves of a growing apple tree pass down through the stem in a rather straight course with comparatively slow lateral movement. Roberts (21) has made use of this fact in explaining some phases of growth as related to the method of preparing grafts. When this food reaches a place in its downward flow where the direct channel is stopped, and where lateral transfer is difficult, it tends to accumulate. Such a situation, for example, occurs in a cutting or in a

recently planted graft on which the lower cion tip extends beyond the cut on the root (Pl. XLIII, C, a). When this happens under suitable environmental conditions, rapid cell division is stimulated. Ordinarily the exposed surface of the cambium proliferates most rapidly and forms the well known Leyden wedge and a considerable portion of the callus overgrowth. The influences of temperature and, even more particularly, of moisture are of very great importance in determining the extent of callus development. In moist soil or in moist packing material where drying is precluded, a considerable development of soft callus tissue may occur. The surface of this tissue is granular, and growth occurs primarily by the division and enlargement of the surface cells. After some time a phellogen develops, woody deposits occur in the walls of some of the interior cells and a cambium is differentiated which produces the usual tissues on either side. Root primordia may develop, and in some cases the enlargement may be covered with a large number of small roots which give the appearance of one type of hairy root. In some cases the small roots appear to grow together and give the overgrowth the aspect of a ball of knotted string. Wound overgrowths have been observed to reach considerable size on some plants and to continue their development for several years. Wounds above ground on apple trees form very little soft callus tissue because of the drying influence of the atmosphere. Such wounds are covered slowly by the overgrowth edge as described by Sorauer (28). Injuries such as those from cultivators, hoes, hooks, and insects may serve to stop the downward passage of elaborated food and to lead to developments similar to those which follow a poor fit in grafting. Such developments are normal processes of the plants when suitable conditions are provided.

The lower tip of the cion is a particularly important place in relation to the development of callus. This has already been observed in relation to the very common position of the knot on the nursery trees, and to the position of the callus on the lower tip of the cion. This relationship was still more apparent when the cions were cut to a feather edge and permitted to callus without having a root attached. Two hundred cions were cut in this manner, placed in new moist sawdust such as is used for packing grafts, and stored at temperatures varying from 10° to 15° C. for two months. At the end of that time all but two had developed active callus around the cut surface of the cambium. By far the greatest development had occurred at the sharp lower end (Pl. XLIV, A). At this place the cell proliferation had been so great that a considerable mass of callus tissue had developed, which in most cases was as large in diameter as the original cion wood. Cultural examinations of these enlargements were negative for the crowngall organism. It seems probable that the food as it descended the twig to the cut made a slow lateral movement along an obtuse angle to the lower tip where

it accumulated and contributed to the development of callus. In a well fitted graft (Pl. XLIV, C) such an accumulation occurred in a much smaller degree because the food material passed through the uniting cambium layers into the root. However, as may be seen in Plates XLIII, G, and XLIV, B, this formation of callus is common where the root and cion are not properly united.

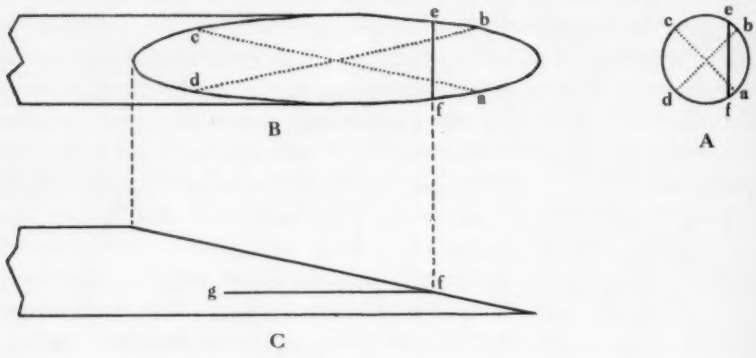

FIG. 2.—Diagram of the lower part of the cion of a commercial piece-root apple graft.

The fit of the lower tip of the cion is important also because there is less cambial surface exposed and therefore less opportunity for union on this sector of the graft than on the side. This is shown in figure 2, which was prepared from a representative commercial apple graft. Here A represents an end view, B a front view, and C a side view of the slanting cut on a cion. Lines *ef* and *fg*, respectively, represent the slit in the cion tip across the front and down the side, into which the tongue of the stock is to be inserted. Suppose that the cion should be quartered by planes which coincide with the lines *ac* and *bd* so that the extreme tip is included in the quarter bound on the outside of the curve *ab*. Now, since the union between the stock and cion takes place almost completely through the callus initiated by cambium, the sector bounded on the outside by the curve *ab* has only the area about this curve in which to unite. However, the sector of the cion bounded on the outside by the curve *ad* has not only the area about *ad* but also that about *fg* and *gf* in which to unite. It should be remembered that in the actual graft the tongue of the root is inserted in the cut represented by the line *fg* (compare with Pl. XLIII). Actual measurements show that the distance *ab* is less than one third of $ad + fg + gf$. So far as cambial surface is concerned, the chances for union are just as small for the sector bounded on the outside by the curve *cd* as for that bounded by *ab*. However, in actual practice the stock is usually cut so that any callus which develops

about the cambium represented by *cd* soon comes in contact with callus on the stock. At the same time, as the metabolic products come down the stem in the quarter bounded on the outside by the curve *cd*, they may, by slight divergence along an obtuse angle, continue their passage. Such translocation would be much more difficult if not impossible in the quarter bounded on the outside by the curve *ab*.

The influences of temperature and moisture on the development of callus on apple grafts were studied. The results conformed so closely with the well established information on this subject that the details of these experiments are omitted. It was found that within certain limits callus development increased with higher temperatures and moistures and decreased with lower temperatures and moistures. Grafts which had formed comparatively little callus at the time of planting grew better than those which had produced excess callus.

The relation between excess callus development and the fit of grafts was tested in preliminary experiments in the greenhouse. Approximately a hundred commercial grafts taken at random were sorted according to the fit of the lower cion tip and planted in pots. The different classes into which they were sorted have been described. After three months the grafts were all dug and further classified according to the amount of callus that had developed. The results appear in table 2. When six of these callus forma-

TABLE 2.—*The relation of fit of cion and stock to the development of overgrowths on commercial Wealthy grafts grown in the greenhouse for three months*

Fit of cion tip[a]	Type of wrap	Dead	Size of callus formed on cion			Total
			None or less than ¼ diameter of cion	¼ to ¾ diameter of cion	Over ¾ diameter of cion	
		No.	No.	No.	No.	No.
Short	Tight	----	2	----	----	2
	Loose	1	3	2	----	6
Even	Tight	----	2	1	----	3
	Loose	5	10	11	----	26
Over	Tight	----	1	----	----	1
	Loose	----	1[b]	9	2	12
Long	Tight	1	----	10	1	12
	Loose	3	----	6	2	11
Blunt	Tight	1	----	2	----	3
	Loose	4	3	11	4	22
Total		15	22	52	9	98

[a] For explanation of classification used see text.

[b] Lower extremity of cion tip dead.

tions were cultured, all were negative for the crowngall organism. These data indicate that grafts which have the lower cion tip cut in such manner that its cambium fails to make contact with that of the root are more likely than well fitted grafts to produce excess callus. Furthermore, the larger amount of callus which developed on properly fitted but loosely wrapped grafts emphasizes the value of tight wrapping. This seems necessary to prevent the lower cion tip from being pushed away from the stock by the development of a cushion of callus tissue.

The importance of the fit of the cion was further tested in preliminary experiments in which grafts were made with care to secure types of good fit and of poor fit at the lower cion tip. At the same time precautions were taken to exclude *Bact. tumefaciens*. The roots were thoroughly washed and the cion wood and roots were dipped in mercuric cyanide, 1–1,000. The knives were dipped before each cut in mercuric chloride, 1–1,000. These antiseptics were employed because Reimer (14) had found them to be very effective in his work with fire blight. The grafting was done in a transfer chamber to minimize chances for contamination. The grafts were wrapped with ordinary unwaxed No. 18 cotton thread which had been autoclaved. The hands of the operators were dipped in mercuric chloride, 1–1,000. After the grafts were made they were planted in soil which had been placed in ordinary greenhouse flats and autoclaved on each of three successive days for five hours at 15 pounds pressure. The pots used were new and had been dipped in mercuric cyanide, 1–1,000. After the grafts were planted they were placed in a greenhouse where there were no plants with crowngall. The experiment was begun in February, 1925. Twenty of the grafts were accurately fitted. After two months all were alive and vigorous, and none of them had developed excess callus (Pl. XLIV, C). Fifty grafts were made with the lower cion tips long. After two months four were dead, four had decayed lower cion tips, and 42 had well developed masses of excess callus (Pl. XLIV, B), with the characteristic granular appearance. Cultural examinations were made of the interior parts of five of these enlargements, but most of them were sterile and all were negative for the crowngall organism. These callus formations appear to be identical with those which are found on commercial grafts (Pl. XLIII, G). These experiments strongly indicate that excess callus formations typical of those which occur so commonly about the unions of grafted apple trees in the nursery may be developed following misfits in grafting, without the intervention of the crowngall organism.

Field experiments were performed in 1923 in which precautions were taken to exclude the crowngall organism. The roots and cion wood were dipped in mercuric cyanide, 1–1,000, before grafting. Before each cut the knives were treated with mercuric chloride, 1–1,000. Grafts were cut and

fitted as in ordinary commercial propagation and wrapped with untreated cotton thread. They were then placed in clean sand until the cions and stocks were well united by callus. They were planted at Madison, Wisconsin, in soil which had been steamed as described by Johnson (4) for tobacco beds. A railing of boards was placed about the treated soil so as to prevent surface water from running into the plot. Of the 74 trees, which were harvested in the fall after a season's growth, diagnosis based on external characters revealed that 51 were smooth, 16 showed wound overgrowth at the union (Pl. XLV, E), and 7 showed hairy root (Pl. XLV, A, B, C). None showed the symptoms of crowngall. Eight of the trees showing malformations were subjected to bacteriological examination. All were negative for the crowngall organism. The wound overgrowths in this case were quite hard and bore a considerable number of root primordia.

In 1924 a series similar to the above, except that the grafts were not made under aseptic conditions and were planted in unsteamed soil, yielded the following results, based on external characters after one season of growth. Of 677 trees which were harvested 446 were smooth, 225 showed wound overgrowth at the union, five showed hairy root at the union and one showed crowngall on the cion above the union. It is worthy of note that in each of these last two experiments approximately one-third of the total number of trees harvested showed malformations at the union. It is quite common for the nurserymen to find that a third or more of their Wealthy trees bear root knot. These experiments indicate that except for one specimen the crowngall organism was not a factor in the development of the malformations found on these trees.

Similar experiments were performed in the season of 1925. This year the roots but not the cions were dipped in mercuric cyanide, 1–1,000, before grafting. After callusing in moist sawdust they were planted in soil which had been steamed and enclosed by boards as in the earlier experiment. These grafts had been prepared with a considerable variety of fits and misfits at various parts of the union. A detailed account of the relations of the several types of fits to wound overgrowth formation will be given on a later page. Only those trees which were more than a foot high were considered when results were taken at the end of the first growing season. Diagnosis based on external characters showed that, of the 1,008 trees harvested, 455 were smooth, 271 bore small wound overgrowths at the union (Pl. XLVI, D), 280 bore large wound overgrowths at the union (Pl. XLVI, C), and none bore crowngalls. Uniformly negative results from cultural examinations of ten of these malformations conform with the diagnoses made on the basis of external characters.

Small field plantings of commercial apple grafts were made in unsteamed soil. Of the 527 trees harvested at the end of one growing season

and diagnosed on the basis of external characters, 282 were smooth, 126 bore small wound overgrowths at the union, 115 bore large wound overgrowths at the union, and four bore bacterial galls, one on the cion and three at the union. The diagnosis was checked by cultural examinations of eight representative wound overgrowths, all of which were negative for the crowngall organism, and two crowngalls, both of which were positive. The results of these experiments corroborate those of the experiments of the two previous years, indicating that *Bact. tumefaciens* played only a minor rôle in the formation of the knots on the trees examined.

Excess callus followed by wound overgrowth has been induced on older trees by wounding under conditions in which precautions were taken to exclude the crowngall organism. On March 12, 1925, 29 three-year-old Jonathan trees were thoroughly washed and then dipped in mercuric cyanide, 1–1,000. After this a slanting cut which extended approximately half way through the stem was made with a disinfected knife, sufficiently close to the union that the injury would be under the surface of the soil when the tree was planted. The cut slanted toward the top of the tree at approximately a 45° angle. In order to prevent direct healing a thin strip of inert substance was inserted in the cut. With seven trees mica was employed, while with the remaining 22 a small piece of medical adhesive plaster was placed in each cut. The trees were then planted in new pots which had been dipped in mercuric cyanide, 1–1,000. The soil used had been placed in small flats and autoclaved for five hours at 15 pounds pressure on each of three successive days. The potted trees were placed in a greenhouse where no other plants were growing. Six of these trees were examined May 19. Well developed callus (Pl. XLVI, A) was observed at the injury in four cases, while in two cases wound overgrowths had developed. Isolation studies made on these enlargements were negative for the crowngall organism. The remainder of the trees were examined August 26, 1925. With the exception of two which died all showed wound overgrowth (Pl. XLV, D). In two cases where a bud was close to each injury hairy root developed. Although precautions had been taken to exclude *Bact. tumefaciens* from these injuries, cultural examinations of three representative enlargements were made. They were negative for the crowngall organism. The characters of these enlargements were the same as those given previously for the wound overgrowth. In view of the obvious difficulties attendant upon growing apple grafts under aseptic conditions, no attempt was made in these and previous experiments to exclude all microorganisms throughout the period of the test. However, it is considered that the technique used furnished sufficient safeguards against the presence of the crowngall organism to make the possibility of its intervention exceedingly remote.

Similar results have been secured in the field following different injuries. One-year-old trees in four lots of 12 each were injured on the cion above the union but below the ground line. The first lot was bruised with a hammer, the second was given a series of needle punctures, the third was given a deep cut which slanted upward, and the fourth had the same treatment as the third except that adhesive tape was inserted in the cut. The trees were planted and left for an entire growing season. They were in rather poor condition at the time of planting. This factor, rather than the injury given them, is probably responsible for the large number which died. The first lot, which was bruised, produced two smooth trees, two which bore hairy root, and eight which had wound overgrowths. The second lot, which received punctures, produced five trees with small and one with large wound overgrowths. The other six died. The third lot, which was cut, produced five small and one large wound overgrowths. The other six died. The fourth lot, which had tape placed in the cut, produced five medium sized and two large wound overgrowths. The other five died. Similar experiments were tried on three-year-old trees. Twenty-five trees were either bruised as in the first lot above, or cut and treated as in the fourth lot. None of them died. In one season 19 of them produced wound overgrowths which were of such size that they would have been rejected during nursery inspection. Cultural examination of representative enlargements from these experiments were negative for the crowngall organism. These experiments give further evidence that wound overgrowths are developed without the intervention of the crowngall organism.

All the experiments and observations which the writers have made point consistently to the conclusion that, except for a comparatively small percentage which usually had distinctive characters, the enlargements which they studied on apple nursery stock were not caused by the crowngall organism. On the other hand the characters of these malformations made them appear to be identical with wound overgrowths produced by poor grafting and wounding. Consequently their control appears to be most readily approached by modifications of horticultural practices, rather than by efforts to prevent infection by the crowngall organism.

The relation of the fit of the graft to the development of wound overgrowth in commercial practice was studied by sorting commercial grafts obtained as representative of those prepared by certain nurseries into the several classes described earlier and planting them in the field. Approximately half the grafts were planted in steamed soil. At the end of the first growing season an examination based on external characters showed that crowngall had developed on less than one per cent of the trees in unsteamed soil and on none in steamed soil. Cultural examinations of 10 wound overgrowths were negative for the crowngall organism. In view of these facts

the data on crowngall are omitted and those concerning the development of wound overgrowths on both of these plots are merged (table 3). As the trees were dug they were classified as to whether they were smooth or bore wound overgrowths. These enlargements were further divided into groups called "small" and "large" according to the standards shown in Plate XLVI, C, D. The results taken on these trees, which were grown at Madison, Wisconsin, in the season of 1925, are given in table 3. Here it appears that

TABLE 3.—*Correlation between the fit of the lower tip of the cion to the root of commercial Wealthy apple grafts and the development of wound overgrowths during the first season*[a]

Fit at lower tip of cion	Grafts planted	Stand over 1 ft. high[b]	Smooth[c]	Small knot	Large knot
	No.	Per cent	Per cent	Per cent	Per cent
Short	149	59	65	23	12
Even	274	64	79	13	8
Over	207	61	52	29	19
Long	466	54	24	38	38
Blunt	558	61	47	32	21
Average		60	53	27	20

[a] The grafts were hand wrapped with waxed No. 18 cotton thread. The figures include both tightly and loosely wrapped unions. No grafts with cions larger than the stocks were employed. For explanation of classifications used see text.

[b] Six per cent of the trees harvested had small callus formations on the cions, probably due to mechanical injuries.

[c] Eight per cent had wound overgrowths at some location other than the lower tip of the cion. On one per cent the lower tip of the cion was dead.

the harvest from grafts with cion tips "even" contained a smaller number of wound overgrowths than that from any other class. In this class with well fitted unions a still lower percentage might have been expected had all the grafts been tightly wrapped. As has been mentioned earlier the cion tips which are not securely held in place often may be pushed away from the root as the callus develops, even when they are cut to the proper length. The group of grafts with long cion tips (Pl. XLIII, C) yielded the highest percentage of wound overgrowths, while the other groups produced intermediate percentages.

It is to be observed that, although approximately three-fourths of the grafts with long lower cion tips produced trees with enlargements, about one-fourth produced smooth trees. Although much still remains to be learned about the formation of wound overgrowths several rather obvious factors which might account for these smooth trees should be mentioned. (a) It was quite apparent on some of these trees that wound overgrowths did not

develop at the lower tip of the cion because of the death of tissues in this region. However, this would account for only a very small portion of the trees which did not bear enlargements, except in certain cases where the grafts were treated with antiseptics. In one lot of grafts which were prepared with long cion tips and wrapped with unwaxed cotton thread, dipped in a commercial preparation of hydroxymercurichlorophenol, 1–400, and planted in pots in the greenhouse for two months, the lower tips of 50 per cent of the cions were killed. Of the remaining 50 per cent a large majority developed typical excess callus formations at the lower cion tips. The stand was not reduced by this treatment. On control grafts in this experiment death of the tissues of the lower cion tips occurred only in occasional instances. (b) In case good union occurred on both sides of the cion tip the food was apparently transferred slightly to one side or the other and so failed to accumulate in the cion tip. (c) It has been observed sometimes that the callus on the lower cion tip may send out roots very early in its development. In such cases the roots may carry off the food material, and thus prevent its accumulation in the lower cion tip (Pl. XLVII, B). Unfortunately, with the exception of grafts treated with certain antiseptics, these and other influences did not seem to operate on the Wealthy variety to make smooth trees from more than approximately a quarter of the grafts that had long lower cion tips.

The importance of the lower cion tip in production of wound overgrowths was further shown in an experiment in which the wedge graft was employed.The lower end of the cion was sharpened to form a wedge and inserted into a split in the upper end of the stock. In each case the cion was equal to or smaller than the root in diameter. The grafts were wrapped with waxed string and planted in steamed soil for the growing season of 1925. Of 200 grafts planted, 105 were over a foot high when harvested. Only 7 of these showed any wound overgrowth. No crowngall was found. This experiment and those described on earlier pages show that the fitting of the grafted parts, particularly at the lower tip of the cion, is important in relation to the occurrence of wound overgrowths.

The influence of the diameter of the cion relative to that of the stock was tested with a limited number of grafts. The grafts in this experiment all had a difference in diameter between the cion and stock of at least one-sixteenth of an inch. They were planted in the spring of 1925 at Madison, Wisconsin, in soil which had been steamed out of doors by the method used in earlier experiments, and were harvested at the end of the first growing season. The results are shown in table 4. For comparison the average from table 3 is shown as a commercial lot. Here it may be noted that the grafts in which the cion was smaller than the stock (Pl. XLIII, E) produced trees with approximately the same number of wound overgrowths as the

commercial lot. However, the grafts in which the cions were larger in diameter than the stocks (Pl. XLIII, F) produced a much higher percentage of wound overgrowths. This type of fit of necessity left some cambium on the cion exposed in such a manner that union with the cambium on the root was difficult. These results indicate strongly the positive correlation between the fit in which the cion is larger than the stock and the development of wound overgrowth.

TABLE 4.—*Correlation between the relative diameter of cion and stock of commercial Wealthy apple grafts and the development of wound overgrowths during the first season*[a]

Classes	Grafts planted	Stand over 1 ft. high	Smooth	Small knot	Large knot
	No.	Per cent	Per cent	Per cent	Per cent
Commercial[b]	1654	60	53	27	20
Cion larger than stock	100	56	9	27	64
Cion smaller than stock	100	50	46	32	22

[a] These grafts were wrapped with waxed thread. For explanation of classifications used see text.

[b] A sample lot of grafts obtained from a nursery.

The influence of several types of wrapping on the development of wound overgrowths was also studied. The different wrappers were placed on commercial grafts made by the same operators who prepared the grafts which were sorted for the planting referred to in table 3. For this reason the average from table 3 is entered for comparison with the results of this experiment. As described in the preceding paragraph the grafts were planted in steamed soil and left through the growing season of 1925. The results at the end of one growing season are given in table 5. Here it may be seen that with loose wrapping (Pl. XLIII, B) the percentage of wound overgrowth was increased. With cloth, which provided a more complete covering than did the string, the amount of wound overgrowth was decreased from the average. However, it should be noted here that untreated cloth rots more rapidly in the soil than does waxed string. The percentage of wound overgrowth was still more decreased with waxed paper, which did not rot so quickly. With the strongest and most persistent wrapper employed, common medical "ZO" or "Zinc oxide" adhesive plaster, there was the smallest percentage of wound overgrowth. This wrapper held the grafted parts very firmly together, prevented the incorporation of soil particles in the callus and opposed the development of excess callus by a firm

binding action which lasted over the most critical period for the initiation of wound overgrowths. However, it rotted (table 6) before any evident girdling effect was induced (Pl. XLVII, A). The diagnosis of these wound overgrowths was based on external characters and checked by cultural examinations of four representative trees, all of which were negative for the crowngall organism. It appears that a tightly applied wrapper which holds the grafted parts securely together without rotting too quickly or lasting long enough to induce girdling is an important factor in preventing the development of wound overgrowths.

TABLE 5.—*Correlation between the kind of wrapping at the union of commercial Wealthy apple grafts and the development of wound overgrowths during the first season*

Type of wrapper	Grafts planted	Stand over 1 ft. high	Smooth	Small knot	Large knot
	No.	Per cent	Per cent	Per cent	Per cent
Waxed string	1654	60	53	27	20
Waxed string (loose wrap at lower end)	312	52	36	30	34
Adhesive plaster	100	47[a]	94	2	4
Waxed paper	290	73	72	17	11
Cloth wrap	100	57[a]	67	21	12

[a] Planted in comparatively poor soil. In other experiments, grafts which were bound with this wrapper and planted in better soil equalled or surpassed those in commercial plantings.

The efficiency of the adhesive plaster used was apparently not due to any germicidal action. Crowngall bacteria from an agar slant were placed on the adhesive surface of a strip of plaster like that used in wrapping the experimental grafts. The plaster was then placed in a petri dish with some strips of moist filter paper to prevent desiccation. At the end of seven days the bacteria on the adhesive surface were still viable. It seems that the zinc oxide is placed in this plaster to prevent irritation of the human skin, and that nothing in the preparation is intended to exert a bactericidal action.

The durability of several of the more common or promising wrapping materials was tested by winding them on sticks and burying them for different periods in ordinary soil with a moisture content suitable for growing plants. At the end of the experiment the tensile strength of the wrappers was measured with a device set up after consultation with M. E. Dunlap of the United States Forest Products Laboratory. The upper end of the material to be tested was fastened in a clamp, and a container was sus-

pended from the lower end. An evenly applied load of sand was poured into the container through a funnel. The weight necessary to break the material was determined. The results, which are the averages of 10 measurements on each lot of material, are given in table 6. It appears that ordinary cotton thread, cloth, and even raffia rot very rapidly. Waxed string and adhesive plaster retain considerable strength for several weeks. Hydroxymercurichlorophenol seems to have exerted a preservative action on both cloth and raffia. It seems, therefore, that, although various chemical substances have proven of very doubtful value in preventing infection by the crowngall organism, some of them may have a distinct value in preserving wrappers and thus indirectly inhibiting the development of wound overgrowths. Further experiments in this direction appear to have considerable promise.

From the several experiments which have been reported above, it appears that excess callus and wound overgrowths may be induced where precautions are taken to exclude the crowngall organism and that, within limits, they may be initiated or suppressed by modifications in cultural practices. Control of these malformations may be accomplished without the use of anti-

TABLE 6.—*Measurements in kilograms made to determine the average tensile strength of one inch lengths of several apple graft wrappers after different periods of time in soil*

Wrapper	Tensile strength after stated intervals:			
	9 weeks	6 weeks	3 weeks	New material
	kg.	kg.	kg.	kg.
No. 18 cotton thread	Disintegrated	Disintegrated	Trace	0.7
No. 18 cotton thread waxed	Trace	0.5	0.7	0.5[a]
Adhesive plaster ½ in. wrapped without overlapping	Trace	0.8	4.8	10.1
Adhesive plaster ½ in. wrapped with overlapping	1.5	4.3	5.9	10.1
Unbleached muslin ½ in. strips	Disintegrated	Disintegrated	Trace	4.5
Unbleached muslin ½ in. strips treated[b]	Disintegrated	Trace	4.6[c]	.....
Raffia	Trace	Trace	0.5	8.4
Raffia treated[b]	Trace	1.0	2.5	.....

[a] When the thread was first waxed the fibers appeared to slip by one another more easily than in untreated thread. This reduced the strength somewhat until the wax became set.

[b] The treatment with hydroxymercurichlorophenol was as follows: The material left nine weeks was dipped in the solution 5 minutes, dried, and dipped again for 5 minutes. The other material was all given two treatments of approximately 6 hours each.

[c] The cloth strips were a trifle wider than ½ inch.

septies, although the latter appear to have important potentialities in the preservation of wrappers. The following practices seem to be among the more promising for the control of wound overgrowths: (a) using cions equal to or smaller in diameter than the corresponding stocks, (b) proper fitting of grafts, especially at the lower tip of the cion, (c) wrapping tightly, especially at the lower tip of the cion, with some material which remains firm through the critical period of callus formation and then rots before it induces girdling, (d) avoidance of excessive callus formation in storage, and (e) prevention of injuries during cultivation.

RELATIVE PREVALENCE OF CROWNGALL AND WOUND OVERGROWTH

When it was found that crowngall and wound overgrowth are due to different causes and may be distinguished with considerable accuracy, an effort was made to determine the relative importance of these malformations in different regions.

A survey was made for crowngall and similar abnormalities among some of the apple nurseries of the North-Central and the Northeastern United States. Representative nurseries which were growing apple trees were visited and the trees examined as they were dug. Where it was impracticable to examine very large numbers the percentages were secured from counts on several hundred trees taken at random. The results, which are summarized in table 7, show that crowngall is very widely distributed in the area surveyed but that it occurred on a very small percentage of apple trees in most nurseries. In the nurseries surveyed, wound overgrowths were generally of very considerable importance on grafted apple trees, but were of comparatively little consequence on budded apple trees. This difference appeared to be due primarily to the wound overgrowths which developed as a result of misfits in grafting. Practical control of wound overgrowths on grafted apple trees was observed in three commercial nurseries in Connecticut, Pennsylvania, and West Virginia (table 7). In each case the nurserymen explained their results on the basis of care in the preparation and handling of the grafts. All used waxed string wrapping and employed no antiseptic. Cultural examination of representative specimens from a number of the nurseries surveyed gave results which confirmed the field diagnoses. The results of this survey agree with those from the other lines of work reported in this paper in showing that, under the range of conditions covered by these studies, the malformations encountered on the underground parts of apple nursery stock were predominantly wound overgrowths rather than crowngall.

TABLE 7.—*Summary of data from survey for crowngall, wound overgrowth, and similar malformations on certain types of nursery stock, 1925*

State	Nursery	Kind of stock	Propagation	Wound overgrowth	Hairy root (all types)	Crown-gall	Woolly aphis
				Per cent	Per cent	Per cent	Per cent
Conn.	A	Apple	Budded	1	1	½	½
		do.	Grafted	2[a]	1	½	½
		Peach	Budded	Trace	0	9	.....
Ind.	A	Apple	do.	Trace	2	Trace	5
		Peach	do.	0	0	Trace	.....
Iowa	A	Apple	Grafted	38	[b]	Trace	.....
		do.	Budded	2	[b]	Trace	.....
	B	do.	Grafted	25	[b]	Trace	.....
		Cherry	Budded	.....	.....	Trace	.....
		Raspberry	.....	.....	.....	Trace	.....
Md.	A	Apple	Budded	8	12	Trace	Trace
		Peach	do.	0	0	0	.....
Mich.	A	Apple	do.	Trace	2	Trace	Trace
	B	do.	do.	Trace	Trace	Trace	25[c]
		Pear	do.	Trace	Trace	Trace	80[c]
	C	Apple	do.	2	8	1	.....
Minn.	A	do.	Grafted	38	4	2	.....
Mo.	A	do.	do.	40	[b]	Trace	.....
		Cherry	Budded	.....	.....	Trace	.....
		Raspberry	.....	.....	.....	3	.....
Neb.	A	Apple	Grafted	33	[b]	2	.....
		Raspberry	.....	.....	.....	5	.....
N. Y.	A	Apple	Budded	½	2	Trace	3
	B	do.	do.	1	13	Trace	1
	C	do.	do.	½	7	½	3
		do.	Seedling	Trace	40[d]	Trace	5
Penn.	A	do	Budded	Trace	2	Trace	1
		do.	Grafted	3[a]	2	Trace	1
		Peach	Budded	0	0	Trace	0
West Va.	A	Apple	Grafted	10[a]	3	Trace	6
		do.	Budded	2	3	Trace	6
Wis.	A	do	Grafted	28	[b]	Trace	.....
	B	do.	do.	24	[b]	Trace	.....
		Raspberry	.....	.....	.....	1	.....

[a] Special care was taken in the preparation of the grafts from which these trees grew.

[b] Hairy root is included in percentage under wound overgrowth.

[c] The diagnosis is doubtful. These were large overgrowths which did not resemble those usually diagnosed as wound overgrowths, bacterial gall, or woolly aphis injury. Because woolly aphis appeared commonly associated with these enlargements, and cultural studies were negative for crowngall, they have been provisionally placed in this column.

[d] These were budded stock on which the bud died. The hairy root in these cases was practically all of the genetic type.

DISCUSSION

Although the results reported in this paper are in general agreement with those of other workers on this subject, they appear to require some important differences in interpretation. These center largely about the differentiation of wound overgrowths from the crowngalls induced by *Bact. tumefaciens*. Earlier workers appear not to have recognized the nature or the importance of these wound overgrowths on apple nursery stock. Consequently, work with two similar types of abnormalities induced by different causes unavoidably led to much confusion in the interpretation of results.

The bacterial nature of crowngall was established by Smith and his coworkers (24, 25, 26), who showed the causal relationship of *Bact. tumefaciens* to crowngalls on a large number of widely different species of plants. Among many other malformations, they studied those on apple trees, to which, however, they devoted relatively limited attention. The present writers have found a record in this work of cultural examination of only 17 root knots on apple, four of which were "soft" and 13 "hard." Positive results are recorded from all the "soft galls" and four of the "hard galls," and negative results from the remaining nine "hard galls." The description of this material does not enable one to determine how many of the "hard galls" may have been wound overgrowths. In this connection, it is pertinent to recall that wound overgrowth and crowngall sometimes merge in one malformation. When these studies were made, the isolation of the crowngall organism was considered to be more difficult than it is at present. Consequently, at that time the positive results were apparently considered to be sufficient basis for the conclusion that *Bact. tumefaciens* was the cause of all the 17 apple root knots cultured.

With the exception of failure to distinguish between crowngall, excess callus, and wound overgrowth, the results of Hedgcock (2, 3) are in many respects in very close accord with those reported herein. He recognized the importance of the fit of cion and stock, the relation of callus to the subsequent development of enlargements, the failure of many antiseptics to prevent the development of malformations, the value of good wrapping, and many other points for which the reader is referred to his papers. Unfortunately, however, so far as the present writers are aware no adequate bacteriological study of Hedgcock's material was made. Consequently, in his later work he appears to have accepted the prevailing conception that *Bact. tumefaciens* was the cause of the enlargements with which he dealt. With the wide acceptance of the parasitic theory of the cause of these malformations, incentive to careful fitting of grafts appears to have been lessened. Many of the nurserymen have increased the speed in the prepara-

tion of grafts, with the expectation of destroying a considerable percentage of the trees because of crowngall infection.

Interpreting their results in accordance with the prevailing conception that they were dealing with a single disease caused by *Bact. tumefaciens*, Melhus and Maney (8) reported reduction in the occurrence of malformations on apple nursery trees, together with some reduction in the stand, following treatments of grafts with Bordeaux mixture. Similarly, Sherbakoff (23) suggested that in some cases a reduction in the percentage of malformations might follow applications of sulphur to the soil in the nursery rows at the time the grafts were planted.

More recently a revision of the earlier inclusive conception of crowngall on apple trees has been in progress. Brown (1), who was co-author with Smith and Townsend in earlier studies on crowngall, has shown through bacteriological studies that many of the aerial knots earlier attributed to the crowngall organism are not induced by this parasite. The present writers have made cultural examinations of 10 such knots, which were secured from West Virginia and New York, and have obtained like results. Swingle (30) has suggested that, since the knots which Brown cultured were apparently non-parasitic, they might be the result of an inherent tendency of the tree. He has listed many varieties of apple according to the prevalence of these developments. The present writers (17, 18) have reported on differences between crowngall and wound overgrowth, with suggestions concerning methods for the control of wound overgrowth. A detailed account of this work is given in the present paper. Recent reports by Melhus (9, 10) and Muncie (12) corroborate these results with reference to the etiology of wound overgrowth. Furthermore, Muncie (11) has reported that certain types of hairy root of apple are noninfectious.

Wormald and Grubb (32) have reported that galls on young apple trees may be induced by various mechanical and chemical agencies as well as by *Bact. tumefaciens*. They state that crowngall may be controlled by care in handling the stocks and by using grafting wax or Stockholm tar.

Waite and Siegler (31) report control of crowngall of grafted apple nursery stock by means of dippings in a solution of hydroxymercurichlorophenol before planting. These investigators appear to adhere to the earlier conception of crowngall, which included the malformations described herein as wound overgrowths, and report no cultural examination of their material. If further study should reveal that they were dealing chiefly with wound overgrowths rather than crowngall, their data on control would agree very closely with the results of the present writers.

Oppenheimer (13) has reported that dipping of apple and pear trees in a solution of hydroxymercurichlorophenol was ineffective against crowngall

infection, but that puddling the trees in a suitable mixture of this compound, soil, and water gave control. It appears that this writer also employs the term crowngall according to the earlier conception, without distinguishing between the bacterial disease and wound overgrowth. In some of his work, however, he used cultures of the crowngall organism.

The segregation of wound overgrowth from crowngall of apple nursery trees appears to clarify numerous perplexing questions, such as: (a) why treatments with certain antiseptics have failed to give control, (b) why certain types of malformations disappeared, (c) why some of the most vigorous trees in the nursery bore large knots and seemed to suffer no ill effects, (d) why reports of injury due to knots in the orchard were very conflicting, (e) why changes to new soil failed to reduce the prevalence of the malformations, (f) why partial control attended the use of certain wrappers and antiseptics, (g) why the occurrence of malformations was minimized by the practice of budding, (h) why cion wood from trees bearing root knots produced no higher percentage of knots than that from smooth trees, and (i) why the majority of the malformations on apple are so different in appearance from crowngall on apple, peach, and raspberry.

The studies herein reported open the way for a new approach to the problem of controlling malformations on grafted apple nursery stock. Since we are concerned primarily with wound overgrowths, most of which are initiated by misfits in grafting or by wounds in cultivation, control may be greatly furthered by improvement in horticultural practices. While much work remains to be done in the refinement of these practices, it has been shown experimentally and demonstrated independently in certain nurseries that the occurrence of wound overgrowths may be greatly reduced by making well fitted grafts, wrapping them tightly with a suitable material, callusing under favorable conditions, and avoiding wounding in cultivation.

SUMMARY AND CONCLUSIONS

1. Crowngall infection of young apple trees was readily induced when *Bact. tumefaciens* was inoculated into wounds of various types. In several experiments, freshly produced callus on apple grafts and young trees failed to serve as an open infection court for this parasite.

2. Crowngall of apple has definite characters which are similar to those of crowngall of peach and raspberry, but are different from those of wound overgrowths on apple, as described herein. Cultural studies have demonstrated that *Bact. tumefaciens* may easily and consistently be isolated from crowngalls of apple in their various stages of development. Crowngalls are commonly comparatively soft, but may at times be hard. The term "hard gall" is not distinctive.

3. Crowngalls are subject to decay by microorganisms. After rotting the galls, these organisms frequently attack the cortex of the young trees, which may be girdled and killed. Crowngalls may overwinter in a sound condition. *Bact. tumefaciens* has been isolated without difficulty from overwintered crowngalls of apple and of raspberry.

4. In the present writers' experiments, many common antiseptics, including certain commercial preparations of hydroxymercurichlorophenol, have proven ineffective in preventing infection of tomato by *Bact. tumefaciens.*

5. Standardized cultural studies of malformations on 407 apple trees taken from several thousand received as "crowngall" rejects from 22 nurseries in 14 states failed to reveal the presence of *Bact. tumefaciens* or any organism closely similar to it in 86 per cent of the trees examined. The same cultural technique applied to typical crowngall on apple, peach, cherry, and plum isolated *Bact. tumefaciens* from 70 of 73 specimens examined. The malformations on the rejected stock which failed to yield the crowngall parasite possessed certain characters which were different from those of crowngall. Because of the relationship of wounds to their initiation and development, they are called wound overgrowths.

6. Wound overgrowths are shown to occur commonly on young apple trees following certain injuries. On grafted apple nursery stock, most such overgrowths develop incidentally to the grafting wounds. They are most abundant, therefore, about the union of cion and stock. They occur most frequently as an overgrowth of the lower tip of the cion. A small number of wound overgrowths on grafted stock and practically all those on budded stock follow injuries induced in cultivation.

7. Differential characters of crowngalls and wound overgrowths are discussed.

8. Examinations of representative lots of commercial apple grafts revealed a sufficient number of misfits to account for most of the wound overgrowths which develop in the nurseries.

9. Under experimental conditions in which precautions were taken to exclude the crowngall organism, wound overgrowths typical of those found in commercial practice have been developed readily from poorly fitted grafts or following wounds. In the same experiments, well-fitted grafts developed smooth unions.

10. Wound overgrowths have been controlled in experimental plots without the use of antiseptics. Important factors in this control were: (a) avoiding the use of cions of greater diameter than the stocks to which they were fitted, (b) proper fit at the lower cion tip, (c) tight wrapping with a material which formed a sufficiently strong bandage about the union and

maintained its strength throughout the critical period for callusing, but rotted before exercising an injurious girdling effect, (d) prevention of excessive callus development in storage, and (e) avoidance of injuries during cultivation.

11. Wax and certain antiseptics have been shown to exert a marked preservative effect on various wrappers commonly used in grafting work. It is probable that such materials may in this way indirectly play a considerable part in controlling wound overgrowths. Some of the antiseptics employed have also reduced wound overgrowth development by injury to the lower cion tip and callus.

12. A survey made in representative nurseries in the North-Central and Northeastern United States showed that crowngall on apple was widely distributed but was much less prevalent than wound overgrowth. Wound overgrowth on apple was found to be commercially controlled in three nurseries where appropriate grafting methods were used and quite generally where the stock was propagated by budding.

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EXPLANATION OF PLATES

Unless otherwise noted all the apple specimens illustrated in these plates are from the Wealthy variety.

PLATE XXXVIII

Crowngall on apple

A.—Natural infection at the union of a two-year-old grafted tree.

B.—Crowngalls which resulted from inoculations on the cion of a Wealthy apple graft at the time of planting. Photographed after one season of growth. A small gall appears directly below the larger one.

C.—Crowngall which resulted from inoculation on a three-year-old Jonathan apple tree. Photographed six months after inoculation. The wound was made by a cut which extended half way through the stem with an upward inclination. To prevent direct healing a strip of medical adhesive plaster was placed in the cut. Although the stimuli for callus as well as for crowngall production appear to have been present the external characters are those of crowngall. This enlargement should be compared with those shown in Plate XLV, D and Plate XLVI, A.

It is noteworthy that these representative crowngalls are comparatively large in relation to the diameter of the trees, and no roots appear from their surface.

PLATE XXXIX

Wound overgrowths and crowngalls on three-year-old fruit trees

A.—Section through an apple tree showing a wound overgrowth which probably developed after an injury at a. The surface bears root primordia and small roots.

B.—Surface of the wound overgrowth shown in A.

C.—Section at the union of a grafted apple tree showing a wound overgrowth about the lower tip (a) of the cion.

D.—Section through an apple tree showing a wound overgrowth which probably followed an injury evidenced by the dark area at a. Numerous small roots have developed from the surface.

E.—Section through a Bradshaw plum tree showing a crowngall which probably resulted from infection in an injury at b. The soft portion of the gall appears dark because of a brown color due to oxidation which occurs rapidly in such tissue. A considerable area of vascular tissue appears (a), making this specimen of crowngall very hard.

F.—Section through an apple tree showing a crowngall which probably resulted from infection through a wound evidenced by the dark area at b. Vascular elements appear at a, but the outer portion of this gall was comparatively soft. In both E and F no roots appear from the surface of the crowngall proper.

PLATE XL

Surface and sectional views of wound overgrowths at the union of three-year-old grafted apple trees

A.—Surface view of the union. Roots and root primordia are present on the surface.

B.—Section of the enlargement shown in A. The lower cion tip (b) of the original graft was too long. Small dark spots may be seen (a) which commonly yielded bacteria in cultural examinations. These, however, were not the crowngall organism. Compare with Plate XLII, C. The evidence now available indicates that these bacteria have little if any influence in the development of the enlargement.

C.—Surface view of the union. Roots and root primordia are present on the surface.

D.—Section of the enlargement shown in C. The lower tip of the cion (b) extended beyond the cambium (a) on the stock and wound overgrowth developed. Small

cavities and fissures (c) may be seen which often contained soil particles and in culture yielded bacteria which seemed not to be pathogenic.

PLATE XLI

Sectional and surface views at the union of one-year-old apple trees

A.—Section showing the relative positions of the lower cion tip (a) and the exposed cambium of the stock (b) of the original graft. The cion tip has been pushed away from the stock by a cushion of callus tissue.

B.—Section through a smooth union. The lower cion tip has been pushed slightly away from the stock by the cushion of callus (a). Here it may be seen that the upper tip of the stock (b) may be long without any significant influence on the development of wound overgrowth.

C.—Similar to A.

D.—Surface view of a tree similar to that shown in A.

E.—Surface view of a tree similar to that shown in B.

F.—Surface view of a tree similar to that shown in C.

PLATE XLII

Photomicrographs of crowngall and callus tissue of apple. Material of both kinds was collected approximately six weeks after the malformations were initiated

A.—Section through crowngall tissue showing that no definite bark layer is formed at the surface (a) but that the outer part may be covered by dead cells. Two separate layers of cells suggesting cambium may be seen, one deeper in the gall than the other, at b and b′. Enlargement appears to occur by growth of inner tissue.

B.—Section through crowngall tissue showing regions of hyperplasia (a and a′).

C.—Section through a small cavity such as may be found both in crowngall and in callus tissues. Such cavities may contain soil and bacteria which appear to have little if any influence in the development of the enlargement. See Plate XL, B, a, D, e.

D.—Section through part of a callus showing parenchymatous tissue, the cells of which later develop secondary thickening in the walls.

E.—Section through callus tissue showing that the surface layer is comprised of living and actively growing cells (a). Later a phellogen and cambium are differentiated and growth appears to take place by secondary thickening such as occurs in stems.

PLATE XLIII

Surface views of the unions of different types of inferior commercial apple grafts

A.—The cion tip (a) is "short." The exposed cambium of the cion does not extend down as far as that on the root.

B.—The cion tip (a) is "even." The exposed cambium of the cion is opposite that of the root. However, in these cases, due to the type of cut and wrapping, the two cambial layers are not held in close contact. The wrapping thread (b) passes under the lower cion tip and hinders contact of the cambial layers. Grafts in which the lower cion tip stands away from the stock are classed as "loose."

C.—The cion tip (a) is "long." The exposed cambium of the cion extends down beyond that of the root. The pin (b) is opposite the lower extremity of the exposed cambium of the root.

D.—The cion tip (a) is "blunt." These grafts were like those shown in C but the long lower cion tip has been cut off.

E.—The cion is smaller than the stock. The lower cion tip (a) is "short."

F.—The cion is larger than the stock. The lower cion tip (a) is "even." A considerable portion of exposed cambium of the cion is not in contact with that of the root. An exposed portion of the tongue is shown at b.

G.—A representative commercial graft like those in C after being packed in moist sawdust for six weeks. Excess callus has developed at the lower cion tip (a).

H.—A graft which shows the development of a number of roots at c on the stock. This may be the early stage of a development of hairy root like that seen in Plate XLV, A, B, C.

PLATE XLIV

The formation of callus on apple cion wood

A.—Cion wood cut to a feather edge and placed in moist sawdust for two months. Callus has developed along the entire cambial surface but particularly at the lower tip.

B.—Grafts with long lower cion tips photographed two months after planting. Considerable excess callus has developed.

C.—Grafts similar to those in B except that the lower cion tip was properly fitted. No excess callus developed.

The grafts shown in B and C were made by a technique designed to exclude the crowngall organism and planted in steamed soil.

PLATE XLV

Malformations which developed on apple grafts and trees planted in steamed soil

A, B, and C.—Hairy root on one-year-old apple trees. These roots originated on the stock. Compare with Plate XLIII, H.

D.—Wound overgrowths and hairy root which developed from slanting cuts in three-year-old Jonathan trees. Photographed six months after the injury. A piece of medical adhesive plaster was placed in each cut to prevent direct healing.

E.—Wound overgrowths which developed at the union of poorly fitted grafts. Photographed after one growing season.

Cultures of all the enlargements shown on this plate were negative for the crowngall organism.

PLATE XLVI

Callus and wound overgrowths on apple trees grown in steamed soil

A.—Similar to Plate XLV, D, except that the photograph was taken two and a half months after the injury. The callus had not yet passed into the wound overgrowth stage. This callus was produced under conditions designed to exclude the crowngall organism.

B.—Wound overgrowths at the union of a poorly fitted graft. Photographed one year after planting. The enlargement marked a is at the lower cion tip and that marked b is on the cion above the upper end of the stock.

C.—Wound overgrowth at the lower cion tip (a). This is typical of the large wound overgrowth of tables 3, 4, and 5. This enlargement developed from a graft like those shown in Plates XLIII, C, and XLIV, B. Photographed after one growing season.

D.—Wound overgrowth at the lower cion tip (a). This is typical of the small wound overgrowths of tables 3, 4, and 5. Photographed after one growing season.

PLATE XLVII

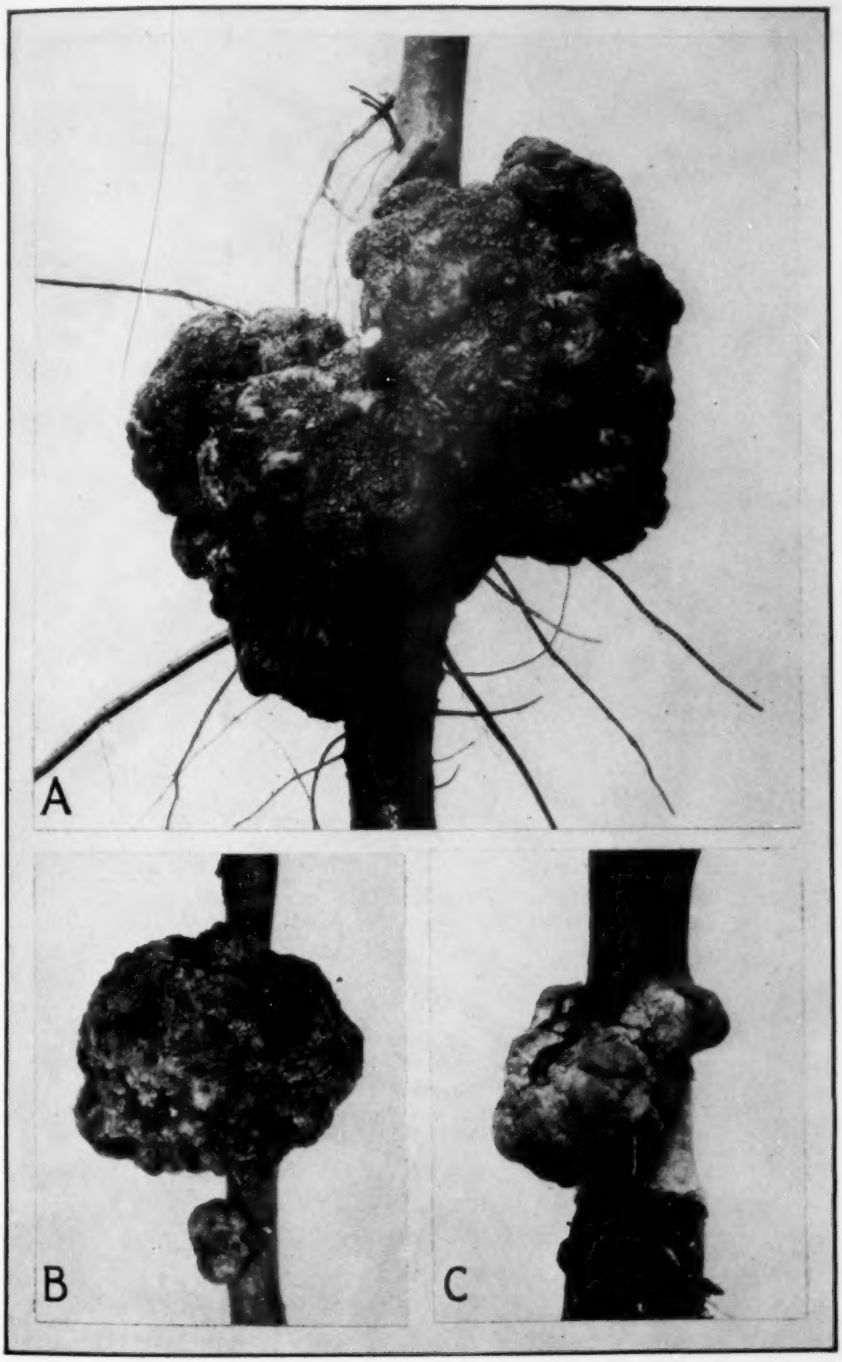
Smooth and malformed unions of apple trees

A.—A smooth union which was wrapped with adhesive plaster which extends from a to b. Roots may be seen piercing the plaster. The cracks in the plaster were produced by expansion of the tree after the plaster rotted. Photographed after one growing season.

B.—Section at the union of a three-year-old tree. The lower cion tip (a) has produced large roots (c and c′) which have assumed much greater importance than the stock (b) in the growth of the tree.

C.—A smooth union on a three-year-old tree. The lower cion tip may be seen at a. In this case the root has developed a greater diameter than the cion.

D.—Hairy root which has developed from the wound overgrowth produced on a graft in which the cion was larger than the root, as shown in Plate XLIII, F. Photographed after one growing season.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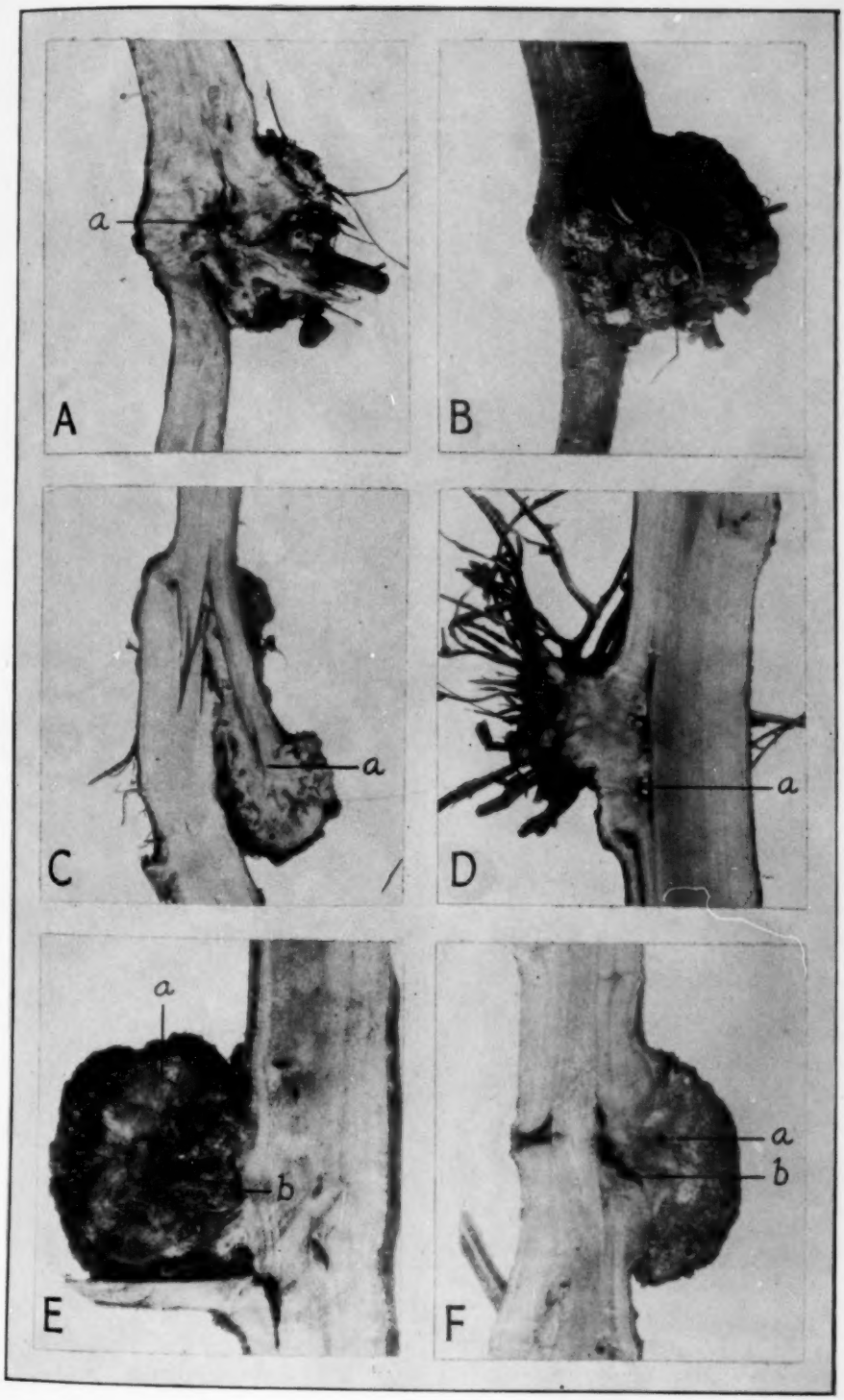
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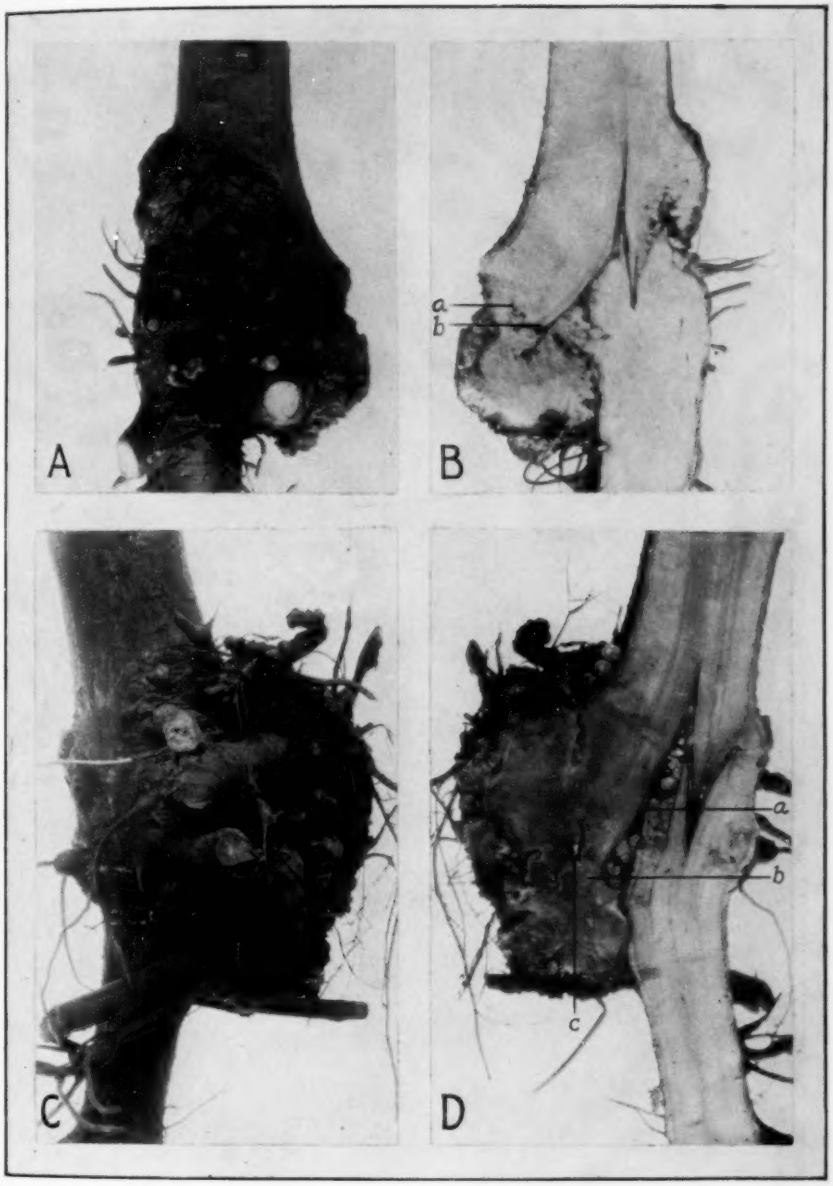
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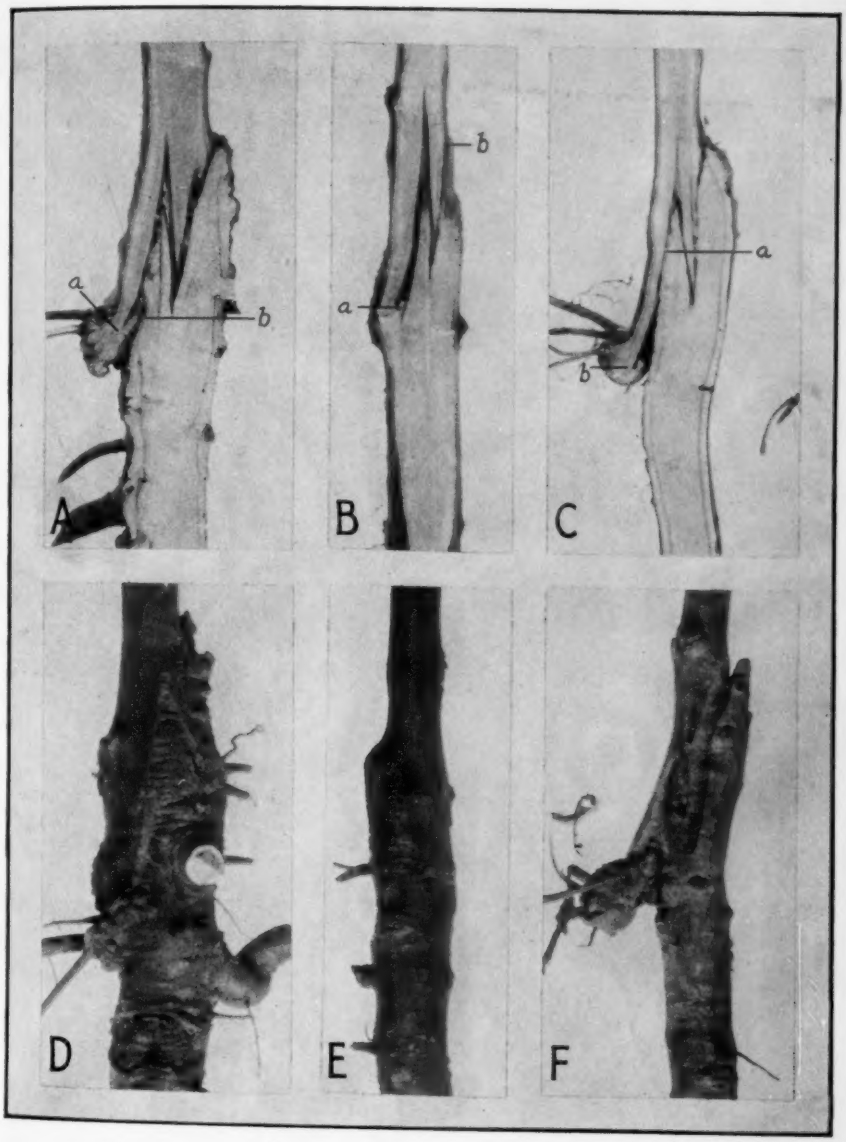
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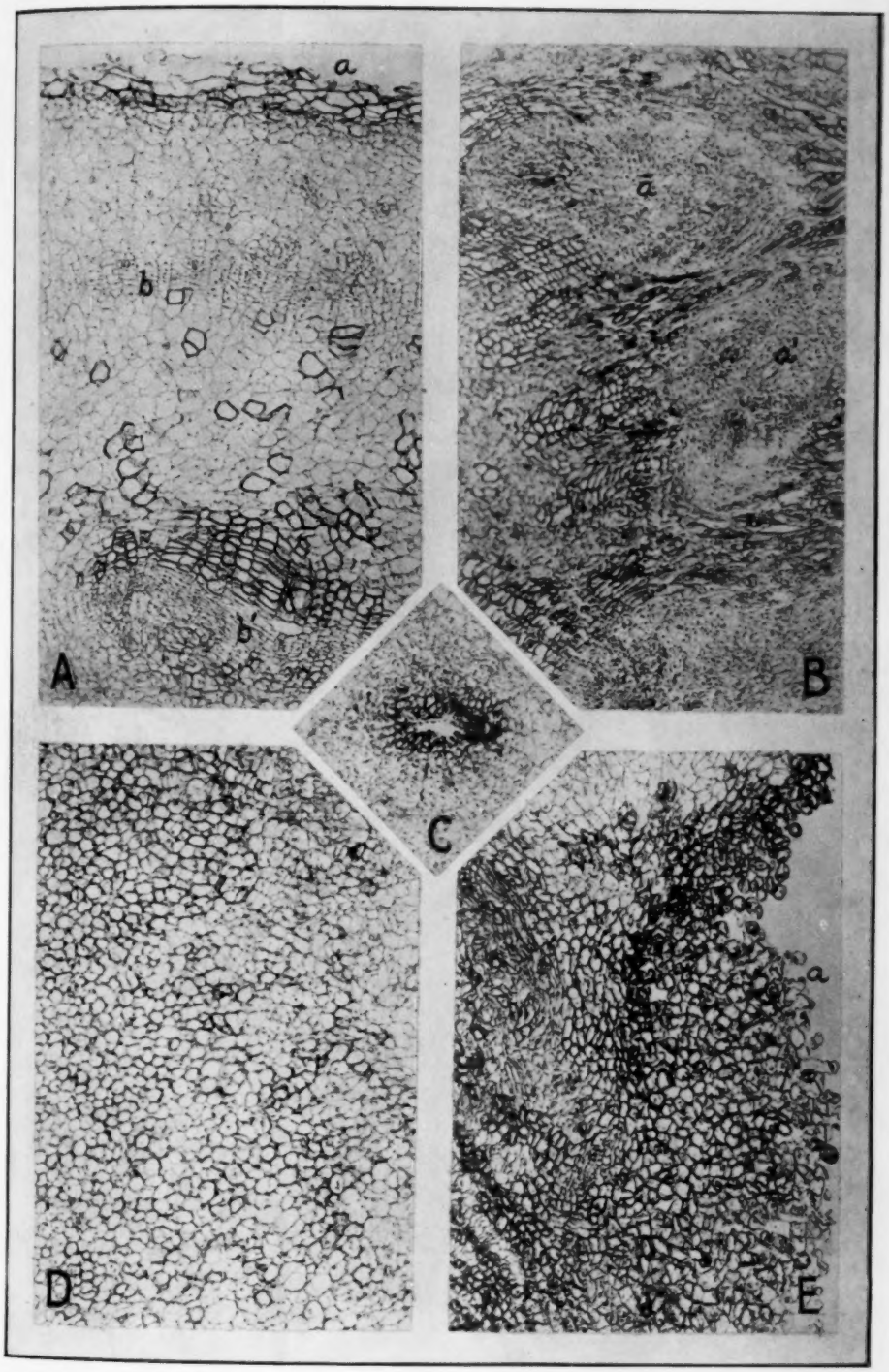
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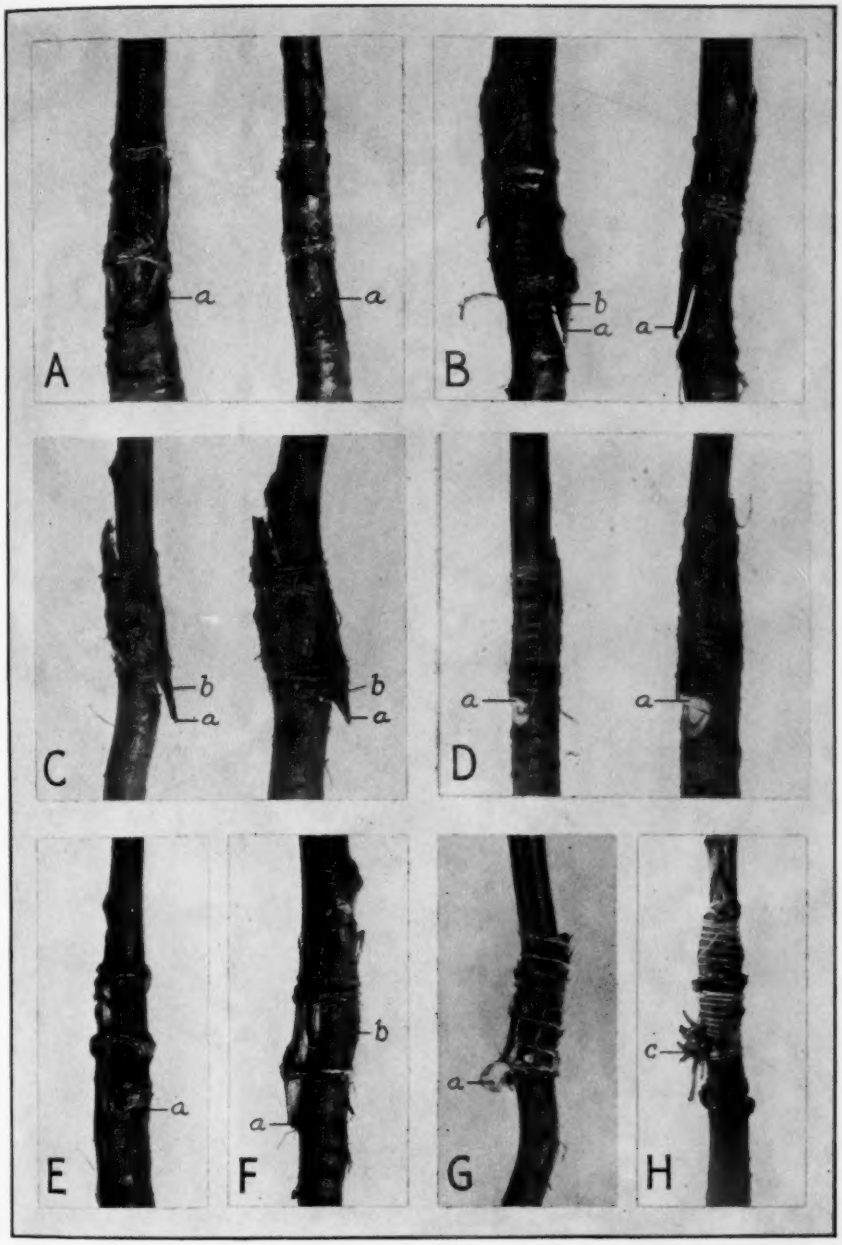
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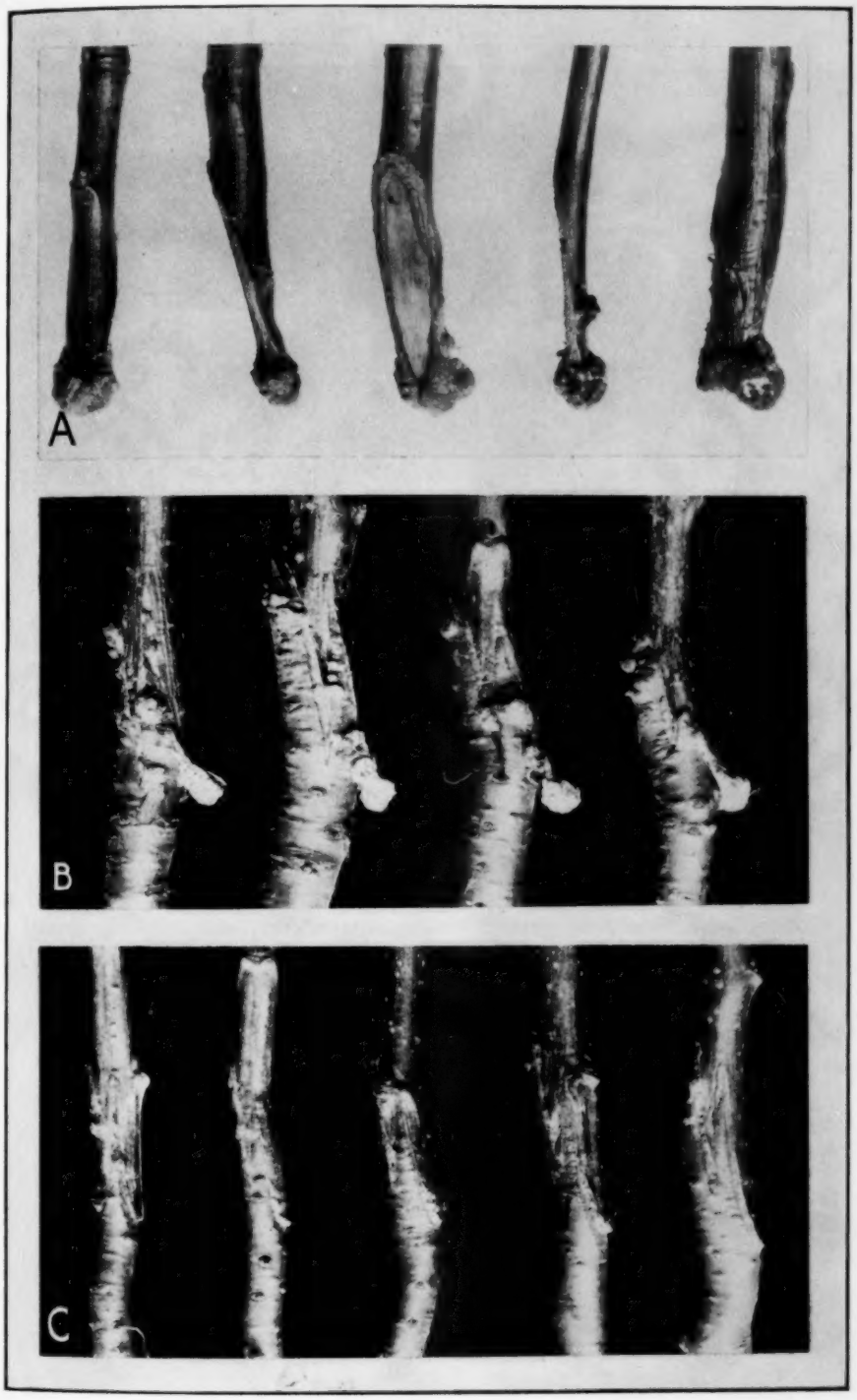
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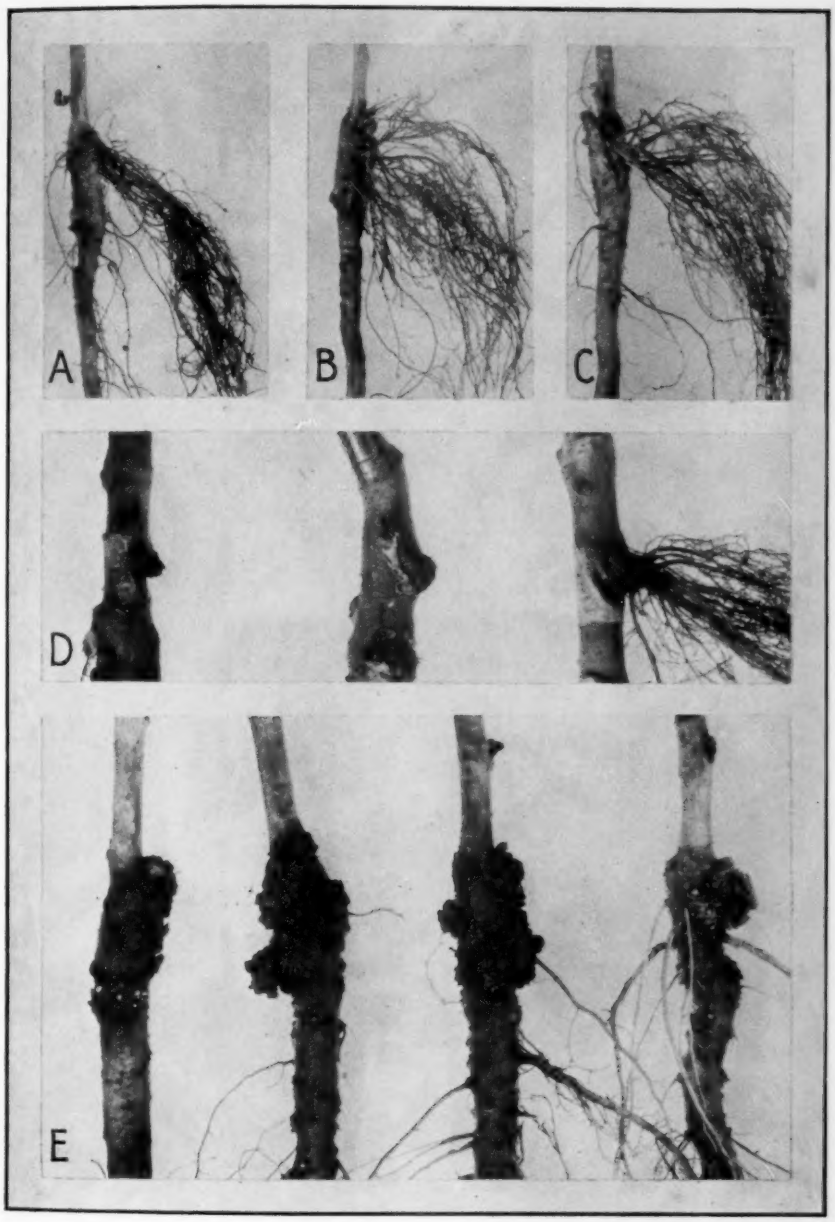
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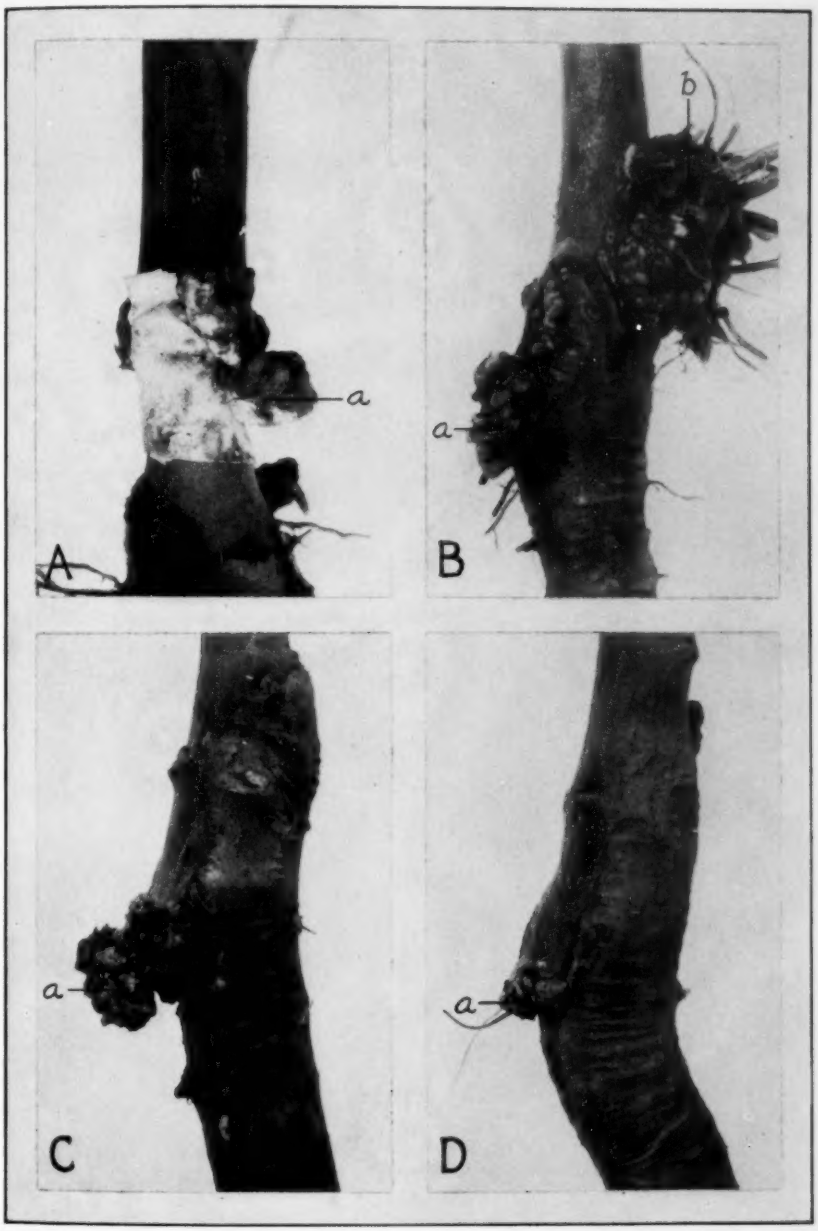
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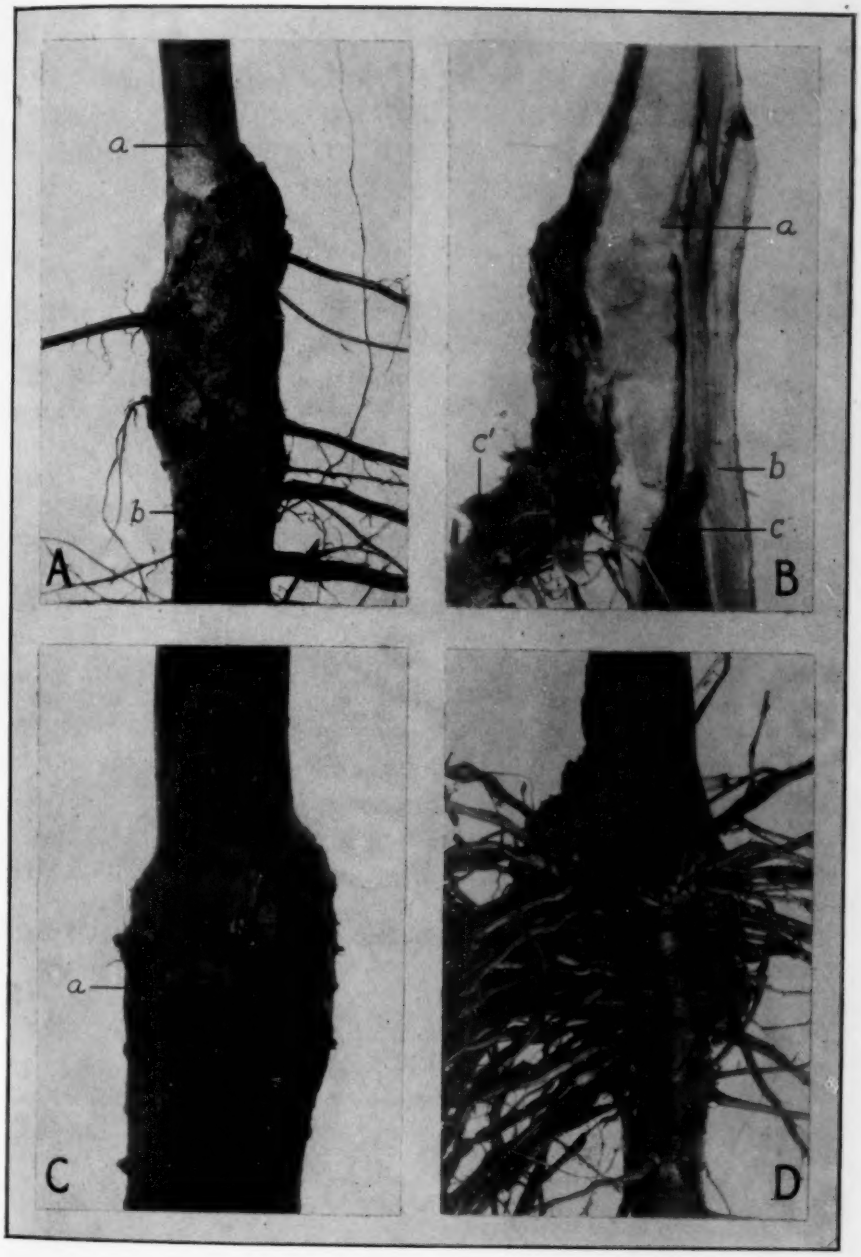
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STUDIES ON THE REACTION TO STEM RUST IN A CROSS BETWEEN FEDERATION WHEAT AND KHAPLI EMMER, WITH NOTES ON THE FERTILITY OF THE HYBRID TYPES

H. J. Hynes[1]

It is generally recognized that the production of varieties of wheat resistant to stem rust (*Puccinia graminis tritici* Erikss. and Henn.) is one of the most hopeful methods of minimizing losses from rust epidemics. This is a complex problem, but investigations on the parasitism of the rust fungus, together with the inheritance studies on resistance to rust in certain crosses, have elucidated the problem considerably.

The extensive researches of Stakman and his co-workers (8, 12, 14, 15) have shown that *Puccinia graminis tritici* consists of 37 or more physiologic forms which can be differentiated by their reaction on 12 varieties of wheat. It is clear, therefore, that if a variety of wheat is classed resistant to stem rust it must be resistant to all the physiologic forms which occur in the particular locality where it is grown. It is evident also that a wheat may be resistant in one region but quite susceptible in another. The problem is still one of developing commercial varieties which are resistant to all forms of rust.

KHAPLI IN RELATION TO STEM RUST

There is only one wheat known, Khapli, C. I.[2] 4013, (15) which is resistant to all known physiologic forms of stem rust. Many attempts (4) have been made to secure a fertile cross between Khapli and Marquis, C. I. 3641, in connection with rust breeding work. These crosses did not appear at all promising, owing to the high degree of sterility and extreme variability of the hybrids.

The present paper deals with studies on a cross between Federation, an Australian mid-season variety of *Triticum vulgare*, and Khapli, an early variety of *T. dicoccum*. The main purpose is to present the results of a study of the reaction of the F_3 generation of the cross to two physiologic

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[2] C. I. = Accession number of the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

forms of *P. graminis tritici*. A classification of the F_2 and F_3 plants, together with the percentage fertility of each, is given in addition to a brief statement on the reaction of certain F_4 families to several forms of rust in the rust nursery, University Farm, St. Paul.

WHEAT SPECIES CROSSES IN RELATION TO STEM RUST

The work of various investigators, Tschermak (16, 17), Kezer and Boyack (6), Freeman (1), Hayes *et al* (2, 5), carried on through the period from 1913 to 1924, contributed results which give a clearer understanding of the species groups in wheat and the difficulties of making successful crosses between them.

Plant breeders are particularly interested at the present time in the possibility of transferring rust resistance from the emmer group to the vulgare group. Freeman and Johnson (4), in 1908, made crosses between varieties of *T. durum* and *T. vulgare* and found it difficult to combine the rust-resistant qualities of the durum parent with the agronomic characters of the common types.

Hayes, Parker, and Kurtzweil (2) studied the inheritance of reaction to stem rust in crosses between *T. vulgare* and *T. dicoccum* and between *T. vulgare* and *T. durum*. They found partial linkage between rust resistance and the emmer and durum types and that it was difficult to secure types which were common and resistant. The fact that some crossing over occurred shows that by growing large numbers of plants in the F_2 and F_3 generations it is possible to obtain rust-resistant common wheat types.

The problem of developing rust-resistant common types from crosses between wheat species of different chromosome number is very complex. In such crosses there is considerable sterility in the resulting progeny. The amount of sterility depends upon the compatibility of the chromosomes of the parents. Cytological studies by Sax (10), Kihara (7), and Watkins (19) have shown that sterility in wheat species hybrids may be due in part to gametic sterility resulting from the proportion of univalent to bivalent chromosomes and in part to zygotic sterility which may result in complete failure of the zygote to function. The possible elimination of certain gametic or zygotic combinations lessens the possibility of obtaining desirable types from species crosses.

The more recent work on wheat species crosses has demonstrated a correlation between chromosome numbers and essential species characters. From his studies on crosses between varieties containing 42 chromosomes (*T. vulgare*) and varieties containing 28 chromosomes (*T. durum*), Sax (11) concludes that it is practically impossible to transfer rust resistance from the emmer group to the vulgare group, since durum characters are

strongly correlated with the 28 chromosome number. In other words, the seven additional univalents contributed by the vulgare parent are responsible for the parent characters, and, since susceptibility to rust is a vulgare character, all segregates which have 42 chromosomes must also be susceptible to rust.

There is evidence to show that this conclusion is not of general application. In the Marquis × Iumillo cross Hayes *et al* (5) have shown that the rust resistance of the durum parent was transferred to a type which had 42 chromosomes and also the characters of the vulgare group. Similar results were obtained in Marquis × Mindum and Marquis × Velvet Chaff crosses. From a cross of Marquis × Iumillo with Marquis × Kanred it appeared that two chromosomes or parts of chromosomes which contained factors for resistance were obtained from the durum parent and were combined with chromosomes of the vulgare group.

These results indicate the possibility of transferring the resistance to stem rust from the 28-chromosome group to the 42-chromosome group. This of course must be difficult since resistance is not only determined by several factors, but chromosome irregularities enter in, which make the obtaining of homozygous individuals of the desired genetic constitution much more difficult.

MATERIALS AND METHODS

In the spring of 1921 the writer crossed Federation wheat (*Triticum vulgare*) and Khapli emmer (*T. dicoccum*). For this work, plants of the parent varieties were made available by Mr. W. L. Waterhouse in the experimental plot, Sydney University, New South Wales, Australia. The grain from which the sowings of these varieties were made had been secured from Mr. J. T. Pridham, Plant Breeder at the Experiment Farm, Cowra, and was labelled "pure line" seed.

The usual technique was employed in making the crosses (3). As a result of artificially pollinating about 15 emasculated flowers of Federation wheat with pollen taken from the Khapli parent, five somewhat shrivelled grains were produced. The F_1 plants, four in all, were quite normal and produced heads bearing plump grain. The heads were intermediate for those characters which separate the parental types. The F_2 grain was also sown in the experimental plot at Sydney University, and in December, 1923, 42 F_2 plants were harvested. Some plants died in the early stages, and others lived until the early boot stage and then died. Studies on the F_2, F_3, and F_4 generations were made at the Section of Plant Pathology, University of Minnesota.

The F_2 generation consisted of plants of very different types which were classified with the assistance of Dr. H. K. Hayes of the Section of Plant

Breeding (Plates XLVIII and XLIX). The percentage fertility was then determined on the basis of the number of seeds set per spike. A portion of the grain from each plant was reserved for planting in the field at University Farm, St. Paul, while the remainder was sown in the greenhouse in connection with a study on the reaction of the F_3 generation to two physiologic forms of stem rust.

The soil used for the greenhouse experiments was steam sterilized before potting in order to avoid injury to the seedlings by root-rotting fungi. Five seeds were sown to a pot. The seedlings were grown during the summer of 1924 in a greenhouse in which there were no rust-infected plants. When they were about 3 inches high, they were inoculated with urediniospores of form 15 and then incubated in a moist chamber for 48 hours. At the end of this time they were placed on well-lighted greenhouse benches where the temperature and moisture conditions were kept as uniform as possible. The methods used in inoculation were the same as those employed by Stakman and Piemeisel (13). Control plants of the parent varieties were inoculated at the same time as each set of hybrids.

Notes on the types of infection with both physiologic forms were taken from 18 to 20 days after the date of inoculation. It was found that environmental conditions influenced to some extent the amount and rapidity of rust development, but the character of infection was always fairly constant regardless of external factors.

After notes on the infection produced by the first form had been recorded, the infected leaves were removed and the plants thoroughly sprayed to remove any remaining spores. These plants were then set aside, and, when the next leaf emerged, were inoculatd with form 27. After incubation they were placed in an adjoining section of the greenhouse where there were no rust-infected plants. Thus there was practically no chance of accidental contamination.

A higher percentage of infection was obtained with form 15 than with form 27. This apparently was due to essential differences between the first and second leaves rather than, as Puttick (9) suggests, to the difficulty of keeping a film of moisture on the second leaf during the incubation period. With special attention a completely saturated atmosphere was maintained during this period. On removal from the incubator there was a film of moisture over the entire leaf surface.

In recording the types of infection the symbols prepared by Stakman and Levine (15) in their work on physiologic specialization were used (Table 1).

TABLE 1.—*Explanation of symbols used to indicate types and degrees of infection produced by physiologic forms of Puccinia graminis on varieties of wheat*

Types of infection

0—IMMUNE

No uredinia developed; hypersensitive flecks usually present, but sometimes there is apparent absolutely no trace of mycelial invasion in the host tissues.

1—VERY RESISTANT

Uredinia minute and isolated; surrounded by sharp, continuous, hypersensitive, necrotic areas.

2—MODERATELY RESISTANT

Uredinia isolated and small to medium in size; hypersensitive areas present in the form of necrotic halos or circles; pustules often in green, but slightly chlorotic, islands.

3—MODERATELY SUSCEPTIBLE

Uredinia medium in size; coalescence infrequent; development of rust somewhat subnormal; true hypersensitiveness absent; chlorotic areas, however, may be present.

4—VERY SUSCEPTIBLE

Uredinia large, numerous and confluent; true hypersensitiveness entirely absent, but chlorosis may be present under certain conditions.

X—HETEROGENEOUS

Uredinia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation possible; on reinoculation small uredinia may produce large ones, and vice versa. Infection ill defined.

Degrees of infection

(=)—TRACE

Uredinia very few in number; development of rust generally poor and decidedly subnormal.

(−)—SLIGHT

Rust development below normal, but somewhat better than "trace."

(±)—MODERATE

Variation in rust development from "slight" to "considerable"; when infection is uniform but only medium in quantity the symbol is omitted.

(+)—CONSIDERABLE

Infection better than normal; uredinia fairly numerous and scattered.

(;)—FLECKS

Hypersensitiveness with no uredinia.

In the rust reactions recorded for the hybrid material the symbols n–, n, and n+ were included under each degree of infection in the moderately susceptible and very susceptible classes. This referred to the degree of necrosis; n– indicated very little necrosis; n, moderate necrosis; and n+, very pronounced necrosis.

PHYSIOLOGIC FORMS USED

In June, 1924, there were about 14 physiologic forms of *P. graminis tritici* available in the greenhouse. Although the reaction of Khapli, C. I. 4013, to these forms was known, it was necessary to know the reaction of Federation, C. I. 4734, before selecting forms for test on the hybrids. Table 2 gives the average types of infection obtained with several forms on Federation and Khapli.

TABLE 2.—*Reaction of Federation and Khapli to ten physiologic forms of Puccinia graminis tritici, expressed by type of infection produced*

Variety	Type of infection produced by physiologic form									
	1A[a]	3A[a]	9C[a]	15	17A[a]	19	21	27	33	38
Federation	4–	4	4–	4 +n-	4	4	4+	4 –n+	4+	4+
Khapli	1–	1	1	1=	1–	1–	1–	1+	1	1–

[a] Single spore cultures kindly made available by Dr. M. N. Levine. Arabic figures indicate form numbers; capital letters designate different monosporous cultures.

It was not possible to select forms which reacted reciprocally on the parents of the hybrids, since Khapli is resistant to all known forms. Forms 15 and 27 were selected. The former is strongly parasitic on all the "differential hosts" except Khapli; the latter is a less virulent form.

Form 15 was collected on wheat at Pusa, India, in March, 1923. Form 27 was likewise collected in India in February, 1923, and was definitely identified in October of the same year. When used in the present investigation, both forms had been cultured through approximately 20 urediniospore generations without any indication of a change in their parasitic behavior.

BASIS FOR INTERPRETING RUST REACTIONS

The results obtained were interpreted largely on the basis of the reactions of the parental varieties to forms 15 and 27. It was considered that all plants which gave a reaction of from 0 to 3= were resistant; plants which showed a high degree of hypersensitiveness in the 3+ classes were placed in the moderately resistant class. In fact it seems reasonable to assume that plants falling in the 3+ class with sharp necrosis were just as resistant as those falling in the 3 or 3– classes without necrosis. Admit-

tedly the line of demarcation between susceptibility and resistance is an arbitrary one, but for the purpose of separating resistant and susceptible families from the plant breeding standpoint the scheme adopted seems to be warranted.

EXPERIMENTAL RESULTS

THE F_2 PLANTS OF FEDERATION × KHAPLI

The data taken on the F_2 plants are summarized in table 3. The hybrids were classified on the bases of prominence of keel, compactness of head and brittleness of the rachis. As already indicated, the percentage fertility was determined on the basis of the number of seeds set per spike.

The F_2 plants were either of the emmer, durum, vulgare or intermediate types. The percentage fertility varied considerably, ranging from 0 to 100 per cent. These results are in accord with those obtained by other workers on emmer-vulgare crosses. The F_1 is usually partially sterile, while in the F_2 variation occurs between complete fertility and complete sterility; the plant characters are those of the parent varieties, or intermediate between them.

TABLE 3.—*Classification and percentage fertility of individual F_2 plants of Federation × Khapli*

Type	Percentage fertility	Type	Percentage fertility	Type	Percentage fertility
Vulgare	37	Intermediate	41	Emmer	6
do	22	do	40	do	6
do	1	do	39	do	5
Intermediate	100	do	38	do	5
do	93	do	26	do	2
do	76	do	17	do	2
do	73	do	8	do	1
do	72	do	4	Durum	59
do	65	do	0	do	58
do	63	Emmer	95	do	46
do	63	do	46	do	40
do	58	do	26	do	39
do	46	do	16	do	24
do	41	do	11	do	3

RESULTS OBTAINED FROM INOCULATING F_3 SEEDLINGS WITH FORMS 15 AND 27

The types of infection obtained on the F_3 seedlings with forms 15 and 27 are summarized in tables 4 and 6 respectively. In general, the percentage of infection obtained was quite satisfactory, although in a few families

there is evidence of little infection. Many plants which gave a "O" reaction from the first inoculation were again inoculated to ascertain whether they were really immune. Many of these proved to be susceptible. A fairly large percentage of plants inoculated with form 27 escaped infection. This apparently was due to the fact that the leaves inoculated with this form had reached a stage of development which was unfavorable for optimum infection. In analyzing the results those plants which appeared immune (O reaction) were disregarded.

A study of the infection data showed a very wide range in type of infection. Some families exhibited practically all types and degrees of infection, together with varying degrees of necrosis. In recording the results it seemed that possibly there were genetic factors which determined pustule development and others, again, which determined the degree of hypersensitiveness. Some plants in certain families developed the parental types of infection, and between these extremes were plants which showed all gradations of infection. These were apparently intermediates. Plants inoculated with form 15 obviously could not give reactions which transgressed those of the parental varieties on account of the extremes of susceptibility and resistance of Federation and Khapli respectively. With form 27, however, some plants appeared more susceptible than Federation. In family 41 there were 6 plants out of 58 infected which were more susceptible than the susceptible parent. This may be a case of transgressive segregation.

Out of a total of 1492 F_3 seedlings inoculated with form 15, 1253 were infected; while with form 27, 527 individuals were infected out of a total of 917 inoculated. The results are summarized in tables 4 and 6. This was done according to the following scheme:

RESISTANT.	Plants showing 0;	to 3 = reactions	inclusive.
INTERMEDIATE.	Plants showing 3 −	to 3 + $^{n+}$ do	do
SUSCEPTIBLE.	Plants showing 3 + ^{n}	to 4 + do	do

Each family was then classified according to the distribution of infection as follows:

S—Homozygous for susceptibility.
HS—Heterozygous, with susceptibility dominant.
H—Heterozygous. Plants evenly distributed in all classes.
HR—Heterozygous, with resistance dominant.
R—Homozygous for resistance.

From table 4 it is seen that nearly half the number of families fall into the H class in their reaction to form 15. Of the remaining families, 10 are classified as HS, and 3 as HR.

In analyzing the data, it appeared that there were two, and probably three, disturbing factors which precluded an exact genetic interpretation of the results. The first was the low percentage of fertility of certain families. In crosses between varieties of *T. vulgare* and *T. dicoccum* considerable sterility results. This might cause the elimination of certain gametic or zygotic combinations, thus probably eliminating the type desired. Another

TABLE 4.—*Summary of results showing the reaction of the parent varieties and F_3 families of Federation × Khapli to P. graminis tritici f. 15 Stak. and Lvne. and the number of individuals in each class*

Parents or F_3 hybrid families	Reaction of family	Number inoculated	Per cent infected	Resistant	Intermediate	Susceptible	Total
Federation	S	60	95	0	0	57	57
Khapli	R	26	88	23	0	0	23
1	HS	328	86	21	49	212	282
2	HS	83	89	1	8	65	74
3	HS	76	87	7	11	48	66
4	HS	47	85	2	6	32	40
5	H	39	79	8	5	18	31
6	H	4	100	0	4	0	4
7	H	33	97	4	16	12	32
8	H	9	100	5	1	3	9
9	HR	16	100	13	2	1	16
10	HS	208	73	11	19	121	151
11	H	21	81	6	1	10	17
12	HS	25	96	1	2	21	24
13	HS	84	94	7	17	55	79
16	H	5	100	2	0	3	5
18	H	6	100	3	1	2	6
21	H	18	100	3	4	11	18
22	H	7	86	0	3	3	6
23	HS	23	91	1	4	16	21
25	H	3	100	1	0	2	3
26	HR	12	58	3	3	1	7
27	H	28	100	12	7	9	28
28	HS	14	64	2	0	7	9
29	H	15	73	4	3	4	11
30	H	108	88	25	34	36	95
32	H	62	98	18	18	25	61
33	HS	59	90	4	18	31	53
34	H	24	29	2	2	3	7
35	HR	16	81	8	3	2	13
39	HR	27	56	10	2	3	15
41	H	92	76	12	27	31	70

factor is the possibility of cross pollination in hybrids. Hayes, Parker and Kurtzweil (2) give evidence of natural crossing which occurred in the F_2 generation of a vulgare × durum cross. This fact emphasizes the necessity for caution in analyzing data. Unless special care is exercised, it is probable that the inheritance of certain characters of the hybrid parents might be inaccurately determined. The small number of families and the comparatively small amount of grain available for the greenhouse tests also added to the difficulty in analyzing the rust data.

A larger number of seeds was sown than seedlings inoculated. This was due partly to the failure of some seeds to germinate and partly to Helminthosporium infection which caused some seedlings to die in the early

TABLE 5.—*Reaction of certain F_3 families of Federation × Khapli to P. graminis tritici f. 15 Stak. and Lvne.*

Family	No. of plants as resistant as Khapli	No. of plants more susceptible than Khapli	Calculated and observed ratios	Deviation / Probable error
1	12	270	15:1 63:1	2.1 5.4
2	1	73	63:1	0.22
3	2	64	15:1 63:1	1.6 1.42
13	2	77	15:1 63:1	2.03 1.03
27	7	21	3:1	0.0
32	11	50	3:1 15:1	1.8 5.6
33	3	50	15:1	0.26
41	3	67	15:1 63:1	1.004 2.7

stages of growth. All soil was sterilized before potting; the infection from *Helminthosporium* presumably resulted from grains which showed "black point."

The interpretation of the results in terms of Mendelian ratios is therefore scarcely possible. In any case it is evident that the number of seedlings available in certain families for the rust tests was much too small for a genetic analysis. For this reason, families with less than eight seedlings

were disregarded in the analysis. It is realized that eight plants is too small a number to indicate definitely the genetic nature of the F_2 parent, yet the results obtained are sufficiently reliable to indicate the number of factors involved. On this basis, then, it is evident from table 4 that the ratio is as follows:

$$1\,S : 10\,HS : 10\,H : 3\,HR : 1\,R$$

The plants in family 9 were for the most part resistant; it is possible that the plant which showed a "4" type of infection may have resulted

TABLE 6.—*Summary of results showing the reaction of the parent varieties and F_3 families of Federation × Khapli to P. graminis tritici f. 27 Stak. and Lvne., and the number of individuals in each class*

Parents or F_3 hybrid families	Reaction of family	Number inoculated	Per cent infected	Resistant	Intermediate	Susceptible	Total
Federation	S	32	100	0	0	32	32
Khapli	R	26	96	25	0	0	25
1	HR	110	21	16	6	1	23
2	H	22	73	1	7	8	16
3	HS	21	67	0	6	8	14
4	H	29	72	5	7	9	21
5	H	31	65	5	10	5	20
7	H	19	63	3	5	4	12
8	H	10	60	2	2	2	6
9	H	9	56	1	4	0	5
10	HR	132	35	28	10	8	46
11	H	15	53	3	3	2	8
12	HS	15	87	1	3	9	13
13	H	57	74	6	19	17	42
16	HR	6	50	2	1	0	3
18	H	5	60	2	0	1	3
21	HS	11	91	1	2	7	10
22	H	7	71	2	2	1	5
23	S	13	77	0	0	10	10
26	H	13	69	4	0	5	9
27	H	23	87	3	8	9	20
28	H	12	92	5	3	3	11
29	H	20	90	11	2	5	18
30	H	97	67	32	20	13	65
32	HR	40	70	17	9	2	28
33	H	54	41	6	6	10	22
34	HR	21	67	10	2	2	14
35	HR	10	60	5	1	0	6
37	H	2	100	1	1	0	2
39	HR	22	59	10	1	2	13
41	HS	85	68	6	4	48	58
42	H	6	67	2	1	1	4

from open pollination. Assuming that this is so and that the family is resistant, the ratio would be 22 susceptible to 1 resistant. This could be explained fairly satisfactorily on a two factor hypothesis. There is a deviation from the expected of 0.56 times the probable error which indicates a good agreement between the observed, 22:1, and the calculated 15:1. The fact, however, that none of the families are homozygous for susceptibility or resistance and that there are all grades and intergrades of infection in certain families precludes the probability of such a type of inheritance. The results are explained on the basis of multiple factors.

A study of certain F_3 families in regard to their reaction to form 15 is given in table 5. Considering that susceptibility or an intermediate type of infection is dominant over the Khapli type of resistance, and arranging the material in two classes: 1, as resistant as Khapli, and 2, more susceptible than Khapli, it is possible to approximate roughly the number of genetic factors involved. While this method is somewhat questionable, it gives some idea of the complexity of the results.

TABLE 7.—*Reaction of certain F_3 families of Federation × Khapli to P. graminis tritici f. 27 Stak. and Lvne.*

Family	No. of plants as resistant as Khapli	No. of plants more susceptible than Khapli	Calculated and observed ratios	Deviation / Probable error
4	1	20	15:1	0.41
5	2	18	3:1	2.3
			15:1	1.02
13	2	40	15:1	0.58
27	1	19	15:1	0.34
33	3	19	3:1	1.8
			15:1	2.1
41	2	56	15:1	1.3
			63:1	1.7

No definite conclusions can be drawn from the data presented in table 5. It would appear, however, that in families 2, 27, and 33 the observed and the calculated agree rather well. In families 1 and 32 the observed ratios seem to agree fairly well with the calculated ratios, 15:1 and 3:1 respectively.

An analysis similar to that given for results with form 15 was made for data obtained with form 27. (See also Plates L and LI.) This is tabulated in table 6. Disregarding families with less than eight plants, the ratio is as follows:

1 S : 4 HS : 12 H : 5 HR : 0 R

In family 1 only one plant out of 23 was susceptible. If this family is considered resistant, the ratio 21 susceptible to 1 resistant approximates a 15:1 ratio. There is a deviation from the expected of 0.48 times the probable error. For reasons previously given, however, the reaction of the F_3 generation to form 27 is interpreted on a multiple factor basis.

Following the scheme adopted in table 5, an analysis was made of the reaction of certain F_3 families to form 27. These results are presented in table 7. It would appear that the observed ratios in each of the families 4, 13, and 27 agree rather well with the ratio calculated, 15:1.

COMBINATIONS OF SUSCEPTIBILITY AND RESISTANCE OF F_3 FAMILIES TO FORM 15 AND FORM 27

The results obtained with form 15 and form 27 for those families which were inoculated with both forms are correlated in table 8. Various com-

TABLE 8.—*The classified reaction of individual F_3 families of Federation × Khapli to forms 15 and 27 of P. graminis tritici*

Reaction to form 27	Reaction to form 15					Total no. of families
	R	HR	H	HS	S	
R						0
HR		35[a], 39	16 32, 34	1, 10		7
H		9, 26	5, 7, 8, 11 18, 22 27, 29, 30	2, 4, 13, 28, 33		16
HS			21, 41	3, 12		4
S				23		1
Total no. of families		4	14	10	0	28

[a] Each number represents the F_3 family number. Families with less than eight individuals are underlined.

binations resulted. No families appeared, however, which were homozygous for susceptibility or resistance to both forms. There were two families

which appeared heterozygous in their reaction to the two forms, with susceptibility dominant. One family reacted as heterozygous to both forms, with resistance dominant. Many families fell into the H class when their reaction to forms 15 and 27 was correlated. Some were heterozygous to one form and heterozygous, with susceptibility dominant, to the other form. One family appeared heterozygous to form 15 and heterozygous, with resistance dominant, to form 27.

Families 1 and 10 were the most promising from the agronomic standpoint. Each of these was classified as heterozygous, with susceptibility dominant, to form 15; and heterozygous, with resistance dominant, to form 27. In both families the plants consisted of vulgare, intermediate, emmer, and durum types but the intermediate type predominated.

THE F_3 PLANT TYPES

A portion of the grain harvested from each F_2 plant was sown in the field at University Farm, St. Paul, in the spring of 1924. Some of the grains failed to germinate while others grew to the "tuft" stage and then died off. From the sowing 371 plants were harvested. These were of various types and showed wide differences in percentage fertility. Of the

TABLE 9.—*Numbers of F_3 plants of Federation × Khapli of different fertility percentages in each of the four wheat classes*

Percentage fertility	Vulgare	Intermediate	Emmer	Durum
0	4	8	28	22
1–5	1	3	8	7
6–10	–	5	9	11
11–15	2	4	8	8
16–20	2	7	7	–
21–25	5	7	4	5
26–30	2	3	7	3
31–35	1	7	3	4
36–40	4	11	5	6
41–45	1	19	9	6
46–50	3	13	4	2
51–55	3	11	3	4
56–60	9	19	2	–
61–65	2	12	1	1
66–70	3	3	3	3
71–75	1	8	–	1
76–80	1	1	–	–
81–85	–	2	–	–
Totals	44	143	101	83

total number of plants, 71 were dwarfs and the remainder of normal type. These dwarfs varied from 8 to 12 inches in height. Some were quite sterile, while in others about 30 per cent of the spikelets produced grain.

A detailed study was made of the morphological characters of each plant and the types classified from the data obtained on the density[3] of the heads, prominence of keel, and percentage of grain which thrashed free of the glumes after being put once through an individual plant thrasher. The percentage fertility was determined from counts made on the number of florets and number of grains set per spike. All determinations made were based on the examination of two representative heads from each plant. The individual plant data which formed the bases of classification is not presented here. It is of interest, however, to know the number of individuals which fell into the various classes and the fertility of each. This information is given in table 9.

Of the 30 F_2 plants grown in the F_3 generation, 15 were intermediates, 7 were durums, 6 were emmers, and 2 were vulgare-like. The progeny of five of the F_2 durum plants bred true for characters of the durum class, though they were not absolutely fixed. Most of the progeny of the F_2 intermediates consisted of types belonging to the emmer, durum, vulgare, and intermediate classes, while the F_2 emmer plants produced progeny of emmer and durum types. A few of the F_2 durum plants gave progeny in the F_3 which consisted of durum and emmer types. No attempt was made to differentiate between the emmer and spelt types. These types were classified together. A study of the figures for density of head showed that both lax and dense types were to be found in the three classes, emmer, durum, and vulgare. The character of the keel on the outer glume and the percentage of grain which had adherent glumes after thrashing, however, served to differentiate these three types.

REACTION OF F_4 FAMILIES TO STEM RUST IN THE FIELD

About 125 F_3 plants which were of intermediate or vulgare type, and which produced good quality grain, were selected to test the reaction of the progeny to stem rust in the field.[4] These tests were carried out in the rust nursery, University Farm, St. Paul, in the summer of 1925, when the seasonal conditions were particularly favorable for the development of rust. Thirteen physiologic forms of *P. graminis tritici* were used to produce the epidemic.

[3] Density of head was calculated by dividing the length of the head in millimeters by the number of spikelets less one.

[4] Twenty-five to thirty grains of each F_3 plant sown.

All of the hybrid material was susceptible, or of a semi-resistant nature which closely approached susceptibility. Some lines, however, appeared to be segregating for a susceptible and semi-resistant type of reaction. The percentage rust development on the hybrids varied from 65 to 100, although one line which was from an intermediate plant appeared semi-resistant and had 50 per cent infection. The Federation parent had on the average 100 per cent rust, while Khapli averaged 5 per cent in the development of rust. The progeny of a number of F_3 emmer and durum plants were also included in the test, and these proved to be quite susceptible. There appeared to be no relation between head type and rust reaction. The results of the F_4 rust tests are presented in table 10.

TABLE 10.—*The reaction of certain F_4 families of Federation × Khapli to thirteen physiologic forms of stem rust in the Rust Nursery, University Farm, St. Paul, showing the number of families in the different wheat classes*[a]

Percentage stem rust		Number of F_4 families			
		Vulgare	Intermediate	Emmer	Durum
50	SR[b]		1		
65	S[c]			1	
	SR		3		
75	S		2	5	
	SR	2	8		
80	S			2	
	SR		2		
85	S	1			
	SR	1	1		
90	S	7	7	7	
	SR	3	8	1	1
95	S		12	1	1
	SR		9		
100	S	24	32	52	6
Segregating			4		

[a] The classification is that of the F_3 parent plants.

[b] SR = semi-resistant.

[c] S = susceptible.

These results serve to emphasize the difficulty of obtaining from species crosses, wheat varieties which are of common type and which are resistant to several forms of rust. Further work is in progress dealing with the reac-

tion of certain F_5 families to the two physiologic forms of stem rust which are present in Australia (18).

SUMMARY

1. In 1921 a successful cross was made between Federation wheat (*Triticum vulgare*) and Khapli emmer (*T. dicoccum*). This was done at Sydney University, New South Wales, Australia.

2. The F_1 generation consisted of partially sterile intermediate types. In the F_2 many different types appeared which showed in varying degrees the characters of the vulgare, emmer, durum, and intermediate groups. Variation occurred between complete fertility and complete sterility.

3. A study was made of the parasitic capabilities of two physiologic forms of *Puccinia graminis tritici* on the F_3 hybrids under glasshouse conditions.

4. Forms 15 and 27 were selected for the tests. These were known to be constant in their parasitic behavior. Federation, C. I. 4734, was susceptible to both forms, and Khapli, C. I. 4013, resistant to both.

5. Thirty F_3 families were inoculated with form 15 when about three inches high. After the infection types were recorded, the first leaf of each plant was removed and the second leaf inoculated with form 27. In analyzing results families with less than eight individuals were disregarded.

6. Some F_3 families showed all gradations between complete resistance and complete susceptibility to both forms of rust. With both forms there appeared all combinations of size of uredinia and degree of hypersensitiveness.

7. The inheritance of resistance and susceptibility in the F_3 generation to forms 15 and 27 may be interpreted on a multiple factor hypothesis.

8. One family appeared homozygous for susceptibility to form 27 although no families were homozygous for resistance to either of the forms. Some families were heterozygous, with resistance or susceptibility dominant; others were distinctly heterozygous, with plants evenly distributed in the resistant, intermediate, and susceptible classes.

9. Combinations of susceptibility and resistance of individual F_3 families to the two physiologic forms were studied. One family was heterozygous in its reaction to both forms, with resistance dominant.

10. Part of the progeny of thirty F_2 plants was grown in the field at University Farm, St. Paul. Of a total of 371 plants harvested, 71 were dwarfs and the remainder of normal type. These plants were classified into emmer, durum, vulgare and intermediate groups on the bases of prominence of keel, density of heads, and the percentage of grain which had adherent glumes after threshing. Some of the F_2 durum plants' progeny bred true for characters of the durum class, while most of the F_2 intermediate and

emmer types segregated in the F_3 generation. All variations in fertility occurred between 0 and 85 per cent.

11. The progeny of over 200 F_3 plants from the vulgare, intermediate, emmer, and durum classes was tested in the rust nursery for reaction to 13 forms of stem rust. Most of the families proved to be susceptible, but some were segregating for a susceptible and semi-resistant type of reaction. The rust development on the hybrids varied from 50 to 100 per cent. The reaction of most families was a susceptible one although some were semi-resistant. There appeared to be no relation between head type and rust reaction.

12. The reaction of certain F_5 families to the two forms of stem rust in Australia is now under investigation.

In conclusion the writer desires to acknowledge his indebtedness to Professors E. C. Stakman and H. K. Hayes, of the University of Minnesota, for helpful advice and criticism, and also to Mr. O. S. Aamodt, of the United States Department of Agriculture, for recording infection notes on the hybrid material grown in the rust nursery, University Farm, St. Paul.

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EXPLANATION OF PLATES

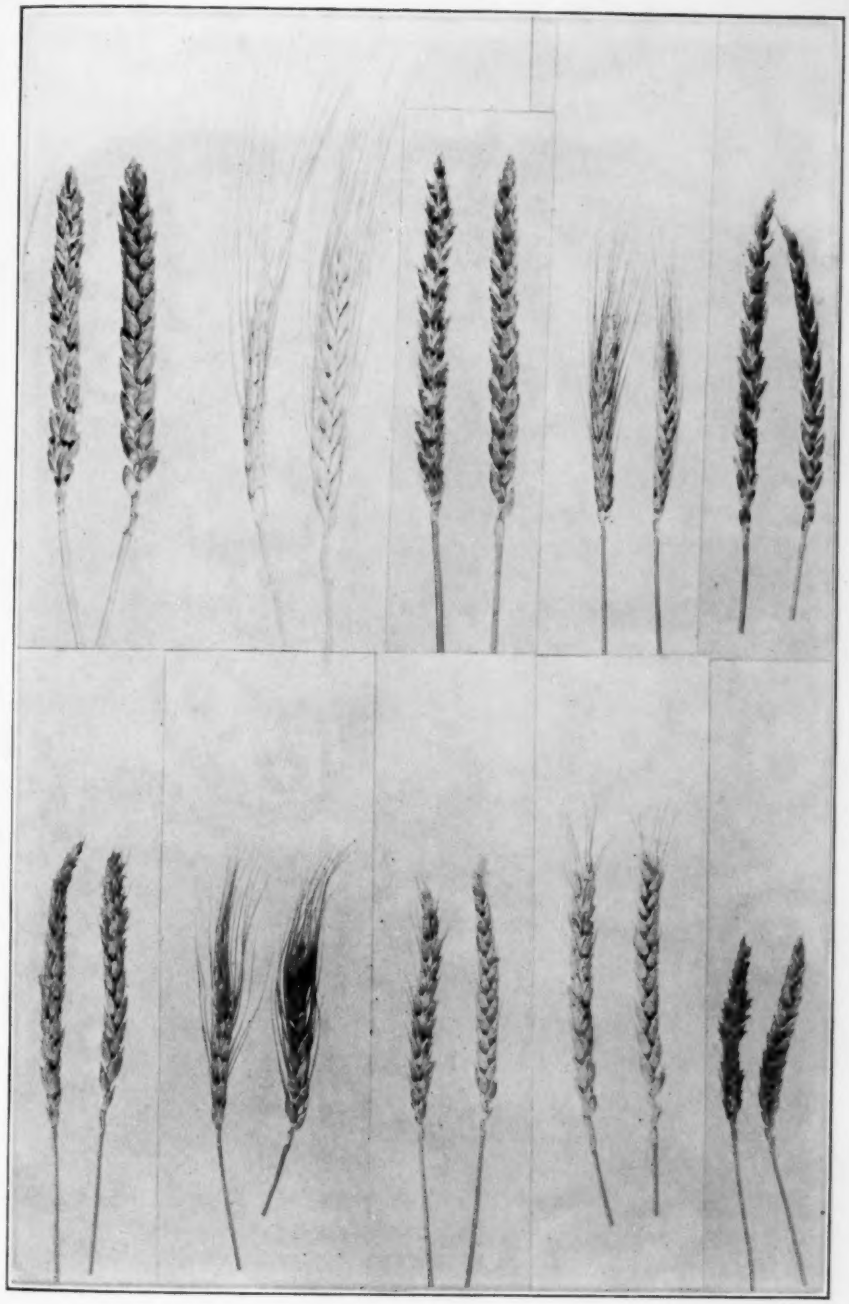
PLATES XLVIII AND XLIX

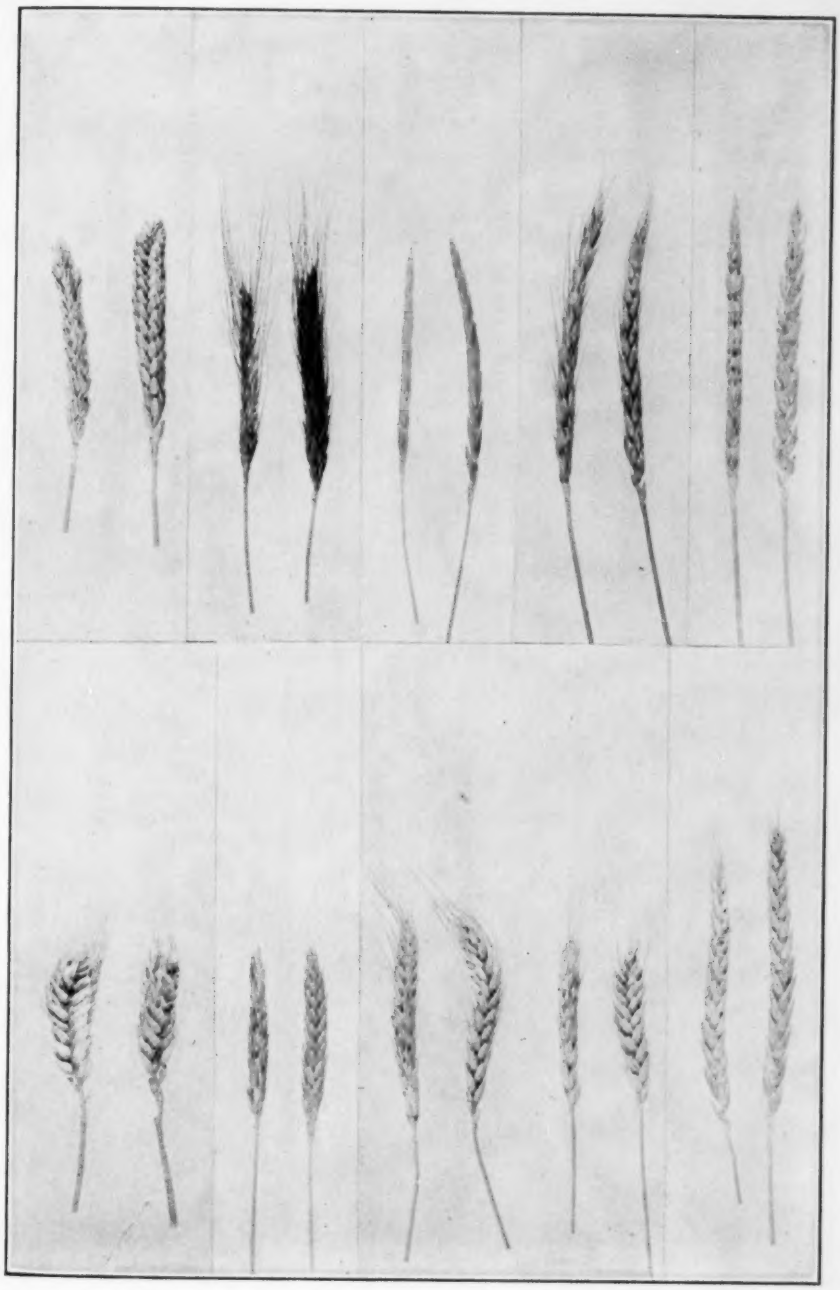
Representative types showing face and side views of F_2 plants of a cross between Federation wheat and Khapli emmer. The first two pairs of heads in the top section of Plate XLVIII are from typical plants of the parent varieties.

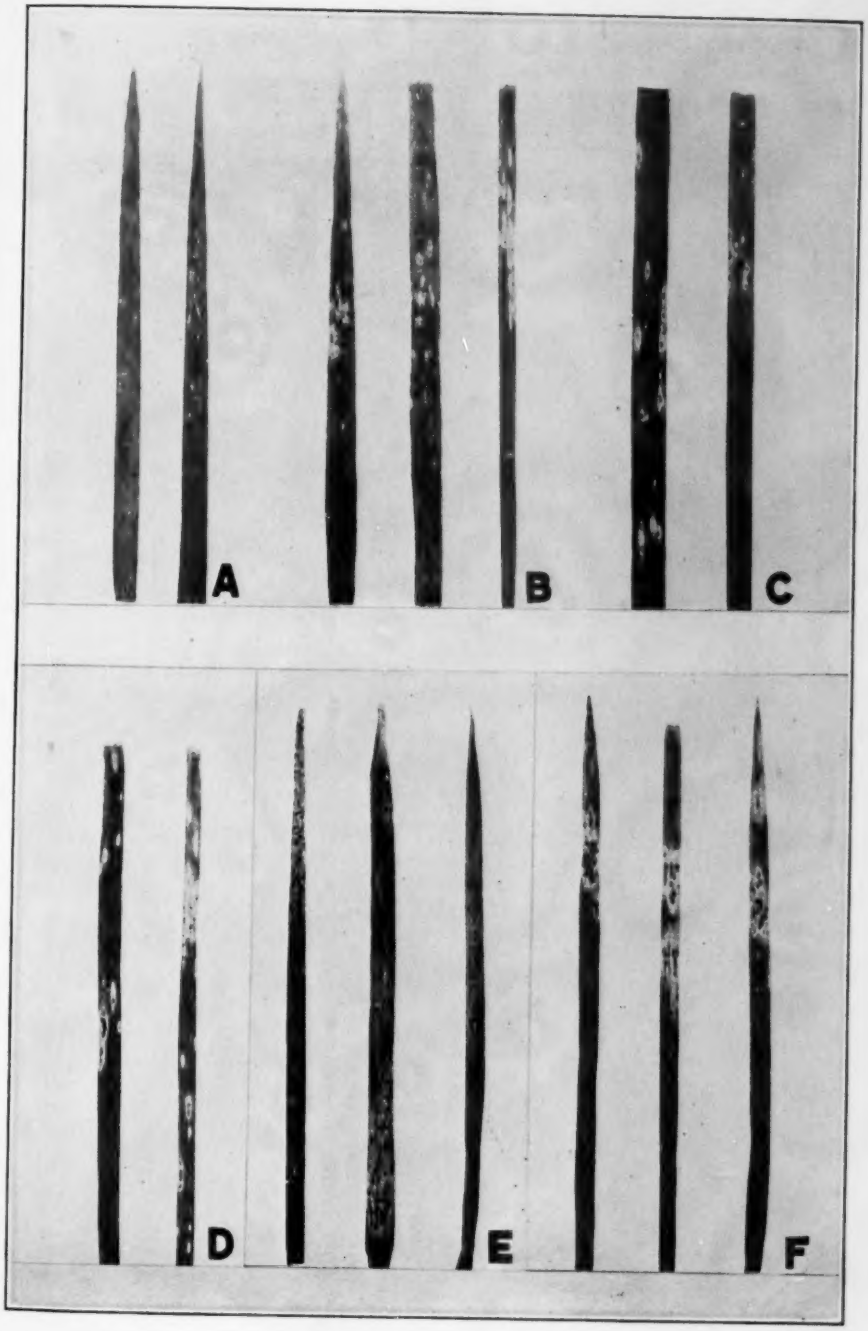
PLATES L AND LI

Reaction of F_3 plants of Federation × Khapli and the parent varieties showing types of infection obtained with *Puccinia graminis tritici* form 27:—

A. 0;
B. 1
C. 2
D. X
E. 3
F. 3^a
G. 4
H. 4^a
I. 1 (Khapli)
J. 4^{a+} (Federation)






A
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E
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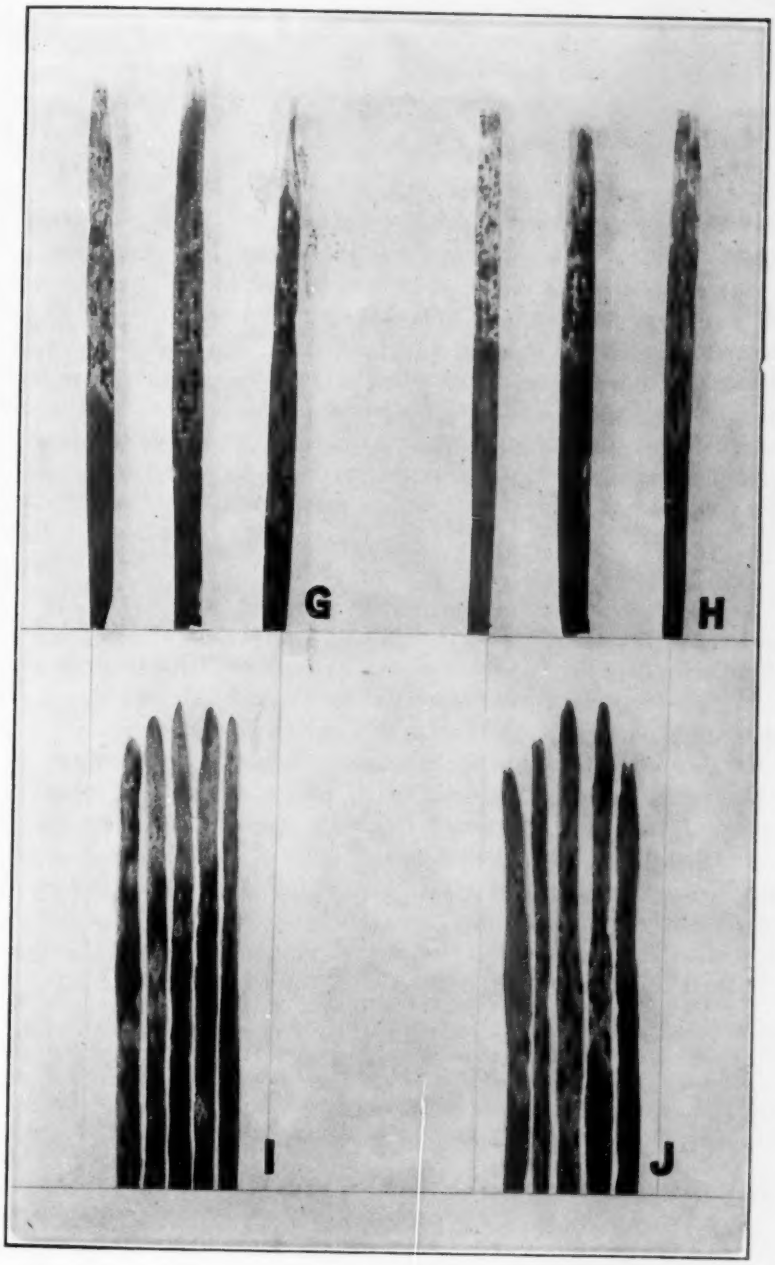
G
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J

SEED TREATMENTS FOR THE CONTROL OF BUNT OF WHEAT[1]

FRED N. BRIGGS[2]

INTRODUCTION

The popularity of the copper carbonate dust treatment for the control of bunt (*Tilletia tritici* [Bjerk.] Winter) in wheat has induced a number of chemical companies and individuals to undertake the production of other chemical dusts to be used for the control of seed-borne diseases, especially those of cereals. At about the time the value of the copper carbonate dust treatment was established in this country a number of organic mercury compounds were introduced for experimental use. From time to time new fungicides, both dust and liquid, have been received and tested for the control of bunt at Davis, California, during one or more years. The data are necessarily fragmentary because all of the fungicides were not available during the entire period and others were dropped after one year because they proved ineffective.

HISTORY

The literature dealing with seed treatment for the control of bunt has become so extensive that no attempt will be made to review all of it in this paper. As early as 1902 Von Tubeuf (9) controlled bunt successfully with copper carbonate dust made in his own laboratory. Apparently no practical use was made of copper carbonate for the control of bunt as a result of Von Tubeuf's experiments. In 1904 Bolley (1) reported an unsuccessful attempt to find a satisfactory dust treatment for the control of bunt. Also, the same year, Wheeler (10) reported that ozonit gas powder, with which he had experimented, would not control bunt. In 1915 Darnell-Smith (2) reported satisfactory control of bunt with copper carbonate, and Mackie and Briggs (4) introduced copper carbonate into the United States for bunt control in 1920. Since that time it has become the most popular treatment

[1] Cooperative investigation between the Bureau of Plant Industry, United States Department of Agriculture, and the Agricultural Experiment Station of the University of California. Throughout the entire progress of this investigation the writer has received many helpful suggestions from Professor W. W. Mackie of the Division of Agronomy, University of California, and from Dr. W. H. Tisdale of the Office of Cereal Investigations.

[2] Assistant Pathologist, Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.

TABLE 1.—*The efficiency of various liquid fungicides for the control of bunt of White Federation wheat at Davis, California*

Treatment	Per cent of Smutty Heads											
	1922 Crop			1923 Crop			1924 Crop			1925 Crop		
	Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Check												
Untreated	47.8	38.4	20.4	55.7	35.3	17.0	61.1	53.1	37.3	63.6	57.3	39.7
Bluestone (1–5)3 min.	.6	0.0	.4	0.0	.5	0.0	.4	0.0	0.0	0.0	0.0	0.0
Bluestone (1–4)3 min.—Lime (1–8)5 min.	1.4	.4	.6	0.0	.3	3.8	6.5	2.1	1.0	4.6	1.8	1.1
Formaldehyde (1–40) 10 min.	.3	.1	0.0	0.0	.9	.6	0.0	0.0	0.0	0.0	0.0	0.5
Solutions												
Chlorophol .1% 30 min.				.6	.4	.3	8.7	4.0	1.1			
.2% do				.6	.2	0.0	1.8	2.9	.2			
.3% do				0.0	.5	0.0						
.1% 1 hour				3.0	.5	.4						
.2% do				.3	0.0	.4						
.3% do	.6	0.0	0.0	.8	0.0	0.0						
Corona No. 620, .05% 30 min.				3.1	2.7	.7						
.1 % do				2.0	0.0	1.0	8.9	4.6	1.4			
.2 % do				1.0	.2	0.0	5.7	.7	1.0			
.05% 1 hour				2.0	.6	1.8						
.1 % do				2.2	.7	.3						
.2 % do				.5	1.0	.5						
Semesan .1% 30 min.				7.0	1.9	1.1						
.2% do				.6	0.0	.5						
.3% do				.2	.3	.5						
.1% 1 hour				2.5	.3	1.2						
.2% do				.2	0.0	0.0						
.3% do				.4	.4	.1						

TABLE 1.—(*Continued*)

	Per cent of Smutty Heads											
	1922 Crop			1923 Crop			1924 Crop			1925 Crop		
	Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Germisan .1% 30 min.				0.0	0.0	.1	14.3	6.5	1.8			
.2% do				0.0	.5	0.0	3.1	.9	0.0			
.3% do				0.0	0.0	0.0						
.1% 1 hour				0.0	0.0	0.0						
.2% do				0.0	0.0	0.0						
.3% do				0.0	0.0	0.0						
Dupont No. 12, .1% 30 min.				1.7	1.0	.8	8.0	3.7	2.1			
.2% do				0.0	0.0	0.0	3.4	1.4	1.1			
Dupont No. 12, .1% 1 hour				1.4	0.0	0.0						
.2% do				.3	.7	.1						
Uspulun .1% 30 min.							14.6	9.0	3.2			
.2% do							4.2	1.5	.7			
Furfural .1% 30 min.							51.0	48.2	30.9			
.2% do							61.2	48.3	36.3			

for the control of bunt in this country because of its effectiveness as a fungicide, lack of seed injury, and ease of application and low labor cost.

Tisdale et al (8) have recently given an account of the development of chlorphenol mercury as a bunt fungicide. It is the basis for such commercial preparations as Uspulun, Chlorophol, and Semesan. Riehm (7) and Remy and Vasters (6) were pioneers in the development of the chlorphenol mercury treatments.

METHODS

A pure line White Federation wheat was used in these experiments because it is a bunt-susceptible wheat and because the smut counts are relatively easy to make in a white-chaffed variety. Three lots of seed were inoculated with spores of bunt, *Tilletia tritici*, with the following proportion of bunt spores to seed by weight: 1:250, 1:750, and 1:1500. The three rates of inoculation were used to secure a better indication of the range of effectiveness of a given fungicide than could be obtained with a single lot.

Duplicate sowings were made in rod rows which were spaced 12 inches apart. One hundred and fifty seeds were sown in each row.

At harvest time the plants were pulled and the total number recorded in order to get an idea as to the effect of the various treatments on the stands of grain. The total number of heads and the smutted heads were recorded and the percentage of smut calculated from these data. A partially smutted head was considered as smutted.

Check rows of untreated seed and seed treated with bluestone, bluestone-lime, formaldehyde, and copper carbonate were included for comparison.

RESULTS WITH SOLUTIONS

Comparatively few solutions were investigated because the farmer prefers a dust treatment, other things being equal. The results obtained with seven different liquid fungicides are recorded in table 1.

The percentage of bunt in the untreated rows was greater in 1924 and 1925 than in the two preceding years. This was due no doubt to climatic conditions at the time of planting. The bunt infection is high enough in each case to give a good measure of the effectiveness of the various treatments. Formaldehyde controlled bunt better than bluestone and bluestone better than bluestone-lime. Absolute control is the goal in seed treatment but many treatments which may be considered entirely satisfactory will not completely eliminate the smut where the seeds are inoculated as heavily as they were in these experiments.

Furfural is the only one of the solutions that did not give satisfactory control and may be dropped from further consideration. A .1 per cent solu-

tion of Chlorophol produced good results in 1923, but in 1924 with a heavier bunt infection the .2 per cent solution produced better results. With Corona No. 620 the results were not quite so good, yet they may be considered satisfactory. A .2 per cent solution of Semesan gave better results than a .1 per cent solution. In 1923 Germisan was outstanding as a fungicide, but the comparatively poor results obtained in 1924 with material from the same supply suggest that it may lose some of its effectiveness on standing. Dupont No. 12 ranks along with Chlorophol and Corona No. 620, while Uspulun is somewhat less effective, being about equal to Germisan in 1924.

The amount of seed injury caused by a given fungicide is very important and is a point which has been given much consideration only during the last few years. In these experiments 150 seeds were sown in each row, and the number of plants present at harvest time was recorded in order to determine the comparative seed injury in each case. While all the plants emerging may not live to maturity, more exact counts may be made at the later date with greater facility. Since the number of plants present at harvest time in each treatment (Table 2) is an average of six rows, it is believed that the comparative figures are reliable.

Bluestone was very injurious to germination in 1922 and 1925. The injury was not so great in the other two years, but the number of plants from treated seed was significantly lower than that from untreated seed. Formaldehyde ranked next to bluestone from the standpoint of seed injury. However, in 1923, formaldehyde caused no injury. The reasons for these seasonal fluctuations with the bluestone and formaldehyde treatments are not well understood. The lime dip following the bluestone treatment prevented most of the seed injury.

Lack of seed injury is oustanding with all of the other solutions used. In 1923 all of the differences were well within three times the probable error as compared with the untreated. Some of the probable errors are very high because the field was infested in spots with morning glory. Seeds treated with Chlorophol, especially the .2 per cent solution for one hour, produced the greatest number of plants. Differences between the other solutions are small and are not significant.

In 1924 seed treated with the five solutions tested produced higher percentages of mature plants than did the untreated seed. The reasons for the greater differences in 1924 than in 1923 are not apparent. The condition of the seed, the soil, the climate, or other factors may be concerned.

Although these new fungicides, except furfural, caused satisfactory bunt control and did not cause any seed injury, in all probability they will not be used for the control of bunt on the West Coast because of the

TABLE 2.—*The number of White Federation wheat plants at harvest from 150 seeds untreated or treated with various fungicides. Average of six seedings at Davis, California*

Treatment	Number of plants at harvest			
	1922	1923	1924	1925
Checks				
Untreated	86.7 ± 1.17	107.2 ± 5.99	95.0 ± 1.08	105.7 ± 1.90
Bluestone (1–5)3 min.	45.8 ± 0.73	82.5 ± 4.86	85.7 ± 2.86	40.0 ± 2.13
Bluestone (1–4)3 min.—Lime (1–8)5 min.	71.8 ± 1.23	105.2 ± 6.25	96.2 ± 1.40	94.3 ± 2.33
Formaldehyde (1–40)10 min.	59.0 ± 1.79	102.0 ± 4.16	58.0 ± 2.89	63.5 ± 0.61
Solutions				
Chlorophol .1% 30 min.		113.3 ± 4.88	119.0 ± 2.80	
.2% do		110.5 ± 4.65	115.3 ± 2.70	
.3% do		105.0 ± 6.14		
.1% 1 hour		109.3 ± 3.80		
.2% do		118.3 ± 1.74		
.3% do	86.6 ± 4.67	112.5 ± 3.09		
Corona No. 620 .05% 30 min.		107.5 ± 2.86	112.5 ± 2.15	
.1 % do		111.5 ± 1.59	116.0 ± 1.35	
.2 % do		100.8 ± 2.86		
.05% 1 hour		106.3 ± 3.80		
.1 % do		104.3 ± 1.93		
.2 % do		98.8 ± 3.51		
Semesan .1% 30 min.		102.3 ± 5.26		
.2% do		103.5 ± 3.02		
.3% do		106.8 ± 5.15		
.1% 1 hour		105.5 ± 5.85		
.2% do		107.3 ± 2.75		
.3% do		98.0 ± 5.58		
Germisan .1% 30 min.		100.8 ± 5.53	114.7 ± 2.90	
.2% do		103.2 ± 4.96	113.3 ± 2.20	
.3% do		103.3 ± 3.14		
.1% 1 hour		105.8 ± 2.78		
.2% do		107.2 ± 1.38		
.3% do		101.7 ± 2.43		
Dupont No. 12 .1% 30 min.		105.8 ± 3.19	120.2 ± 2.64	
.2% do		102.5 ± 3.24	117.3 ± 1.95	
.1% 1 hour		106.5 ± 3.98		
.2% do		106.6 ± 5.44		
Uspulun .1% 30 min.			117.8 ± 1.72	
.2% do			119.8 ± 1.87	

greater ease of application of the dust treatments and the low cost of copper carbonate.

DUSTS

Twenty-four fungicidal dusts other than copper carbonate were investigated in the four-year period. During the first two years the dusts were applied at the rate of from 1 to 4 ounces per bushel. Later the 4-ounce treatment was dropped because with copper carbonate this rate of application was not superior to the 3-ounce treatment and because it was not possible to get this amount of dust to adhere to the seed. The effects of the various dusts on bunt control are recorded in table 3.

The dusts which did not approach copper carbonate in effectiveness and which may be dropped as unsatisfactory are: Seed-O-San, S. D. No. 4, S. D. No. 6, S. D. No. 7, 40–F, 40–S, C-10-F, C-10-G, C-20-F, C-20-G, Karasch compounds A and B, Dupont 8 and 9, Copper Stearate, and Dupont No. 19 A and No. 19 B. Copper carbonate is used as a basis for comparison because it is the only dust treatment that has been extensively used for bunt control and because it has given satisfactory results in the hands of the farmer. In order for a fungicide to replace copper carbonate, it should control bunt as well as or better than copper carbonate, it should be cheaper, and should give stands of plants equal to or better than the stands from seed treated with copper carbonate. Nothing is said about the increase in yield due to seed treatment because we believe that sufficient data are not yet available to warrant any statement.

Coppercarb, a copper compound with less than half the copper content of copper carbonate, produced fairly satisfactory results. However, it takes about twice as much dust per bushel under the conditions of these experiments to equal pure copper carbonate.

Chlorophol is a chlorphenol-mercury compound and was intended by the manufacturers to be used as a solution. However, it gave satisfactory results when used as a dust in 1922. It was not tested further because of its greater cost.

S. D. No. 3 controlled bunt fairly satisfactorily but was not as efficient as S. D. No. 5, which is the concentrated fungicide that forms the toxic principle of the Semesan used in 1925. Semesan and Dupont No. 2 compare favorably with copper carbonate for the control of bunt.

In summarizing the results the data indicate that Chlorophol, S. D. No. 3 and S. D. No. 5, Dupont No. 2 and Semesan controlled bunt approximately as well as copper carbonate dust. S. D. No. 5 gave slightly superior control, especially at the rate of one ounce per bushel.

TABLE 3.—*The efficiency of various dust fungicides for the control of bunt of White Federation wheat at Davis, California*

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Check (untreated)		47.8	38.4	20.4	55.7	35.3	17.0	61.1	53.1	37.3	63.6	57.3	39.7
Copper carbonate	1	22.7	2.2	0.1	12.2	1.5	0.0	17.9	3.5	0.7	32.7	6.8	1.7
	2	9.4	1.2	0.0	4.5	1.3	0.7	5.0	4.1	0.7	13.9	3.0	0.4
	3	1.1	0.4	0.2	0.0	0.0	0.0	4.7	1.9	0.4	4.3	1.2	0.2
	4	2.3	0.1	0.0	0.8	0.0	0.0						
Coppercarb	1				12.3	1.5	2.3	21.6	10.4	2.7			
	2	16.1	5.1	2.5	8.3	2.7	0.3	13.0	6.7	2.6			
	3				7.8	0.1	0.1	8.1	4.6	2.5			
	4	9.9	0.0	0.4	3.6	2.2	0.0						
Chlorophol Dust	1												
	2	19.4	2.4	2.2									
	3												
	4	3.3	0.1	1.6									
Seed-O-San	1				39.9	19.7	11.0						
	2				38.9	6.1	4.6						
	3				32.5	5.5	1.6						
	4				14.6	1.2	0.0						
S. D. No. 3	1				7.5	2.3	0.6	15.4	5.8	1.8			
	2				2.8	0.5	0.0	8.6	1.8	1.0			
	3				1.9	0.5	0.5	4.2	1.3	1.1			
	4				0.7	0.0	0.0						

TABLE 3.—(*Continued*)

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
S. D. No. 4	1				25.9	5.8	0.4						
	2				22.0	5.0	0.0						
	3				5.5	0.5	0.2						
	4				4.6	0.9	0.3						
S. D. No. 5	1				1.3	0.9	0.2	3.0	0.6	1.2			
	2				0.3	0.6	0.0	0.8	0.6	0.2			
	3				0.8	0.0	0.0	0.0	1.1	0.0			
	4				0.5	0.0	0.0						
S. D. No. 6	1				19.0	9.1	7.9						
	2				9.9	7.6	1.2						
	3				3.4	1.5	0.3						
	4				4.5	2.6	0.0						
S. D. No. 7	1				26.7	7.1	4.3						
	2				13.0	4.4	0.2						
	3				5.7	1.0	0.0						
	4				1.8	0.4	0.2						
40–F	1				52.7	22.4	7.0						
	2				33.0	15.4	2.3						
	3				26.9	5.5	0.3						
	4				14.3	2.5	1.1						
40–S	1				40.7	32.7	8.7						
	2				19.2	11.2	1.5						
	3				14.4	3.0	1.4						
	4				9.6	5.4	0.2						

TABLE 3.—(*Continued*)

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
C–10–F	1				46.5	20.7	11.1						
	2				46.4	26.8	7.0						
	3				45.9	20.5	11.1						
	4				35.3	17.7	5.6						
C–10–G	1				35.3	26.7	8.5						
	2				46.9	25.2	8.3						
	3				58.2	18.1	6.7						
	4				39.3	13.2	3.7						
C–20–F	1				41.4	19.2	5.0						
	2				35.9	8.6	1.8						
	3				28.5	8.9	2.6						
	4				9.0	2.8	4.2						
C–20–G	1				33.0	20.3	7.1						
	2				39.0	7.6	4.5						
	3				33.7	2.0	5.0						
	4				16.1	5.4	3.4						
Dupont No. 12	1				21.3	6.5	3.3						
	2				11.3	4.1	1.2						
	3				3.4	0.8	0.8						
	4				4.1	1.3	0.7						
Karasch compound A	1							64.7	50.4	29.4			
	2							58.8	47.6	29.6			
	3							52.6	40.6	24.7			

TABLE 3.—(*Continued*)

Treatment		Per cent of Smutty Heads											
		1922 Crop			1923 Crop			1924 Crop			1925 Crop		
		Proportion spores to seed			Proportion spores to seed			Proportion spores to seed			Proportion spores to seed		
Dusts	Oz. per bu.	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500	1–250	1–750	1–1500
Karasch compound B	1							69.4	46.5	31.6			
	2							60.5	48.8	37.1			
	3							64.2	46.3	29.6			
Dupont No. 2	1							15.2	4.4	2.1	26.4	6.0	1.5
	2							7.0	2.6	2.2	14.0	3.1	1.8
	3							3.9	1.0	0.4	4.8	1.5	0.0
Dupont No. 8	1							52.0	29.7	10.7			
	2							38.0	17.2	6.7			
	3							22.3	16.0	4.1			
Dupont No. 9	1							63.4	25.4	20.2			
	2							49.6	22.1	8.4			
	3							33.9	16.3	2.7			
Copper stearate	1										56.9	31.6	17.0
	2										63.6	30.7	21.2
	3										52.5	32.8	16.4
Semesan	1										22.3	9.7	2.3
	2										10.7	1.2	2.1
	3										12.3	2.2	0.6
Dupont No. 19A	1										69.9	44.6	29.6
	2										65.0	38.1	22.1
	3										67.9	36.8	22.5
Dupont No. 19B	1										62.1	47.5	20.1
	2										56.6	38.1	25.2
	3										60.6	34.5	23.5

The number of mature plants derived from 150 seeds subjected to each of the various dust treatments gives a measure of seed injury, and is recorded in table 4.

TABLE 4. — *The number of White Federation wheat plants at harvest from 150 seeds treated with various dusts. Average of six seedings at Davis, California*

Treatment		Number of plants at harvest from 150 seeds			
Dust	Oz. per bu.	1922	1923	1924	1925
Checks (untreated)		86.7 ± 1.17	107.2 ± 5.99	95.0 ± 1.08	105.7 ± 1.90
Copper carbonate	1	83.0 ± 1.56	93.0 ± 2.45	97.1 ± 2.43	105.5 ± 1.51
	2	85.2 ± 1.79	97.8 ± 4.94	96.3 ± 1.43	109.0 ± 2.64
	3	85.2 ± 3.02	105.1 ± 4.56	101.5 ± 2.39	106.0 ± 1.72
	4	83.3 ± 2.39	100.8 ± 1.79		
Coppercarb	1		112.8 ± 2.41	99.7 ± 2.06	
	2	89.8 ± 1.20	111.1 ± 2.67	103.0 ± 2.32	
	3		110.0 ± 2.67	107.2 ± 0.67	
	4	89.0 ± 1.81	106.7 ± 2.86		
Chlorophol dust	1				
	2	90.7 ± 2.29			
	3				
	4	88.7 ± 1.97			
S. D. No. 3	1		102.0 ± 1.03	113.2 ± 0.95	
	2		105.1 ± 2.77	118.5 ± 3.06	
	3		109.8 ± 4.72	116.7 ± 0.72	
	4		116.5 ± 3.66		
S. D. No. 5	1		106.3 ± 1.93	118.8 ± 2.35	
	2		100.1 ± 4.05	120.2 ± 2.89	
	3		107.8 ± 4.20	128.8 ± 1.72	
	4		109.0 ± 4.61		
Dupont No. 2	1			105.3 ± 2.46	108.7 ± 1.13
	2			112.2 ± 0.99	108.3 ± 2.27
	3			110.2 ± 2.22	105.0 ± 2.39
Semesan	1				107.5 ± 1.30
	2				112.3 ± 1.07
	3				110.6 ± 0.82

In comparing the results of the various treatments with those from untreated seed, it will be seen that there are no significant differences in 1922 and 1923. In 1924 all of the seed treated with S. D. No. 3 and No. 5 and Dupont No. 2 produced stands significantly better than the stands produced by untreated seed while there were no significant differences between

the results obtained from the copper carbonate and coppercarb dust treatments. In 1925, Semesan at the rate of two ounces per bushel was the only treatment superior to the untreated.

As two ounces of copper carbonate dust per bushel of wheat generally is recommended, the stands secured with the other dust treatments may be compared with those from this rate of application. In 1922 none of these treatments was significantly superior to copper carbonate dust in so far as improving stands is concerned. In 1923, S. D. No. 3 at the rate of four ounces produced better results. In 1924, all of the treatments with S. D. No. 3, S. D. No. 5, and Dupont No. 2, and the 3-ounce treatment with coppercarb were superior to copper carbonate applied at the rate of two ounces per bushel. In 1925, there were no significant differences. The reasons for the superior stands in 1924 when the organic mercury compounds were used are not known.

On the whole, the dust treatments caused no seed injury, as evidenced by stands from the treated seed as good as those from untreated seed. From this standpoint they are distinctly superior to the bluestone and formaldehyde treatments which were widely used for many years on the West Coast, especially the bluestone. In more recent years the injury from bluestone was largely prevented by the use of the lime dip. At present copper carbonate is widely used in place of the bluestone and bluestone-lime treatments, and has proven entirely satisfactory both from the standpoint of bunt control and lack of injury to the seed.

SUMMARY

The data presented herein on the control of bunt by liquid and dust treatments were collected over a four-year period beginning in 1922.

In most cases the more promising fungicides were investigated for two years.

All of the liquid fungicides, except furfural, controlled bunt satisfactorily.

Severe seed injury resulted from the use of bluestone. This injury was largely eliminated by the use of the subsequent lime dip. Formaldehyde caused severe injury except in 1923.

There was no injury from the use of Chlorophol, Corona No. 620, Semesan, Germisan, Dupont No. 12, and Uspulun. The stands obtained from seed treated with these compounds were not significantly different from the stands from untreated seed in 1923, but were better in 1924. The reasons for the better stands in 1924 are not known.

Twenty-four dust treatments other than copper carbonate dust were investigated during the four-year period. Of this number only six—Corona Dust, Chlorophol, S. D. No. 3, S. D. No. 5, Dupont No. 2, and Semesan—

controlled bunt as satisfactorily as did copper carbonate. S. D. No. 5 produced slightly better results than copper carbonate, especially with the one-ounce treatment.

Stands of wheat were improved in 1924 by the use of S. D. No. 3, S. D. No. 5, Dupont No. 2, and coppercarb (3 oz. per bu.), as compared with untreated seed and seed treated with two ounces of copper carbonate per bushel. In other years no significant differences were noted.

Liquid treatments are not likely to replace the dust treatments on the West Coast of the United States because of the greater ease in handling the latter.

Of the dusts used, all factors considered, copper carbonate is the most satisfactory.

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EFFECT OF MOSAIC ON CARBOHYDRATE AND NITROGEN CONTENT OF THE TOMATO PLANT[1]

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In order to determine what influence different soil conditions might have upon the severity of the mosaic disease of the tomato, and also what effect the disease might exert upon the growth of the plant and upon its carbohydrate and nitrogen content, tests on potted plants were made in the greenhouse and supplemented by chemical analysis of the plants. Two types of mosaic were tested: typical tomato mosaic, and the necrotic or double-virus type (streak or winter blight).

In addition to one series of plants grown in a bench, two series of pot tests were carried out, one in the spring of 1924 and the other in the fall of 1925. Varnished metal pots of 10 kilograms capacity were used.

TESTS WITH STREAK OR DOUBLE-VIRUS MOSAIC

In the 1924 series, different soil conditions were provided by using a rich potting loam with high, medium, and low water content, and a clay loam very poor in nitrogen and phosphorus to which were added varying amounts of the lacking elements. On March 3, seedlings of the Bonny Best variety were planted in the pots. In this series the streak or necrotic (double-virus) type of mosaic was tested. Johnson (13, p. 10) and Dickson (6) have shown that this type of mosaic is due to the presence of more than one virus, namely, the virus of typical tomato mosaic or Fernow's (10) mosaic A, and a virus from potatoes which is probably the virus of the mosaic designated as B by Fernow (10). Inocula were obtained from two sources: (a) the streak or winter blight type of mosaic in a greenhouse crop of tomatoes, and (b), a necrotically mottled tomato fruit shipped from California to the Chicago market and sent to us by Dr. G. K. K. Link. This inoculum produced a necrotic type of mosaic less extreme than the preceding. For convenience these will be designated as the LaFayette (L) and California (C) types.

The inoculum was prepared by macerating the tissue in a flamed mortar and diluting with sterile water. Inoculation was effected by smearing the

[1] Joint contribution from the Botanical Department, Agricultural Experiment Station, and the Agronomy Department, Purdue University, Lafayette, Ind.

[2] The writers are indebted to Dr. E. G. Campbell and Mr. W. P. Allyn for assistance, and to Professor H. S. Jackson for helpful advice.

stem near the tip with a piece of absorbent cotton dipped in the inoculum and pricking through the smeared area with a flamed needle. On April 10 one plant in each series was inoculated with the LaFayette inoculum, one with the California inoculum, and one served as an uninoculated control.

On May 16, 36 days after inoculation, when the first fruits had set but before any fruits had approached maturity, certain of the plants were taken for fresh weight determination and chemical analysis.

In preparing the material for chemical analysis, the entire plant was weighed, chopped up, and placed in 95 per cent alcohol to which had been added calcium carbonate at the rate of one gram per liter. The pulp was later transferred to weighed beakers which were then placed on an electric hot plate and dried to constant weight. The percentage of dry matter was calculated from the difference in weight. The pulp was reduced to a fine powder and stored in tightly stoppered bottles until analyzed.

For the determination of sugars, a five gram portion of the dry powder was extracted with a 50 per cent alcohol solution in a Soxhlet extractor until all coloring matter had been removed. The solution was then made up to 250 cc. and the reducing sugars were determined by the picric acid colorimetric method (19) after an aliquot had been clarified by mercuric nitrate and sodium carbonate. The non-reducing sugars in this clarified liquid were inverted by the official hydrochloric acid method of inversion (7, p. 186) and determined by the picric acid colorimetric method (19). The polysaccharides in the residue in the extractor were hydrolyzed by the official hydrochloric acid method (7, p. 119) and determined by the picric acid colorimetric method (18). The nitrogen was determined by the Gunning-Kjeldahl method modified to include the nitrates (7, p. 8). The results of these analyses are shown in table 1.

The different soil conditions produced different growth responses as indicated by the differences in fresh weight and chemical composition of the healthy plants. But neither these differences nor differences in the soil moisture content greatly altered the character or severity of the mosaic symptoms on the inoculated plants.

However, there were outstanding and consistent differences between the mosaic and the healthy plants in each series. The mosaic plants were characterized in all cases by a reduction in total fresh weight, in percentage content of dry matter, in carbohydrate content, and in carbohydrate-nitrogen ratio. The reduction in weight of the roots was found to be proportional to the reduction in total weight. The reduction in carbohydrate content was due mainly to the lower content of polysaccharides.

The increase in total nitrogen shown by most of the mosaic plants was more apparent than real because of the lower percentage of dry matter in

TABLE 1.—*Effect of mosaic (streak type) on growth and on carbohydrate and nitrogen content of tomato plants*

Soil	Condition of plant	Fresh weight (grams)	Percentage dry matter	Percentage[1] reducing sugars	Percentage[1] non-reducing sugars	Percentage[1] polysaccharides	Percentage reduction in total carbohydrate content	Percentage[1] total N	Carbohydrate-nitrogen ratio
Rich potting soil	Healthy	657.1	12.7	1.19	1.94	26.8		3.76	7.9
	Mosaic C	419.0	11.4	1.25	1.88	13.5	44.4	3.72	4.5
	Mosaic L	288.0	10.0	1.10	1.70	13.0	47.0	4.36	3.6
P. C. L. + 500 N and 4000 P[2]	Healthy	455.7	14.3	1.25	2.50	38.4		1.45	29.1
	Mosaic C	432.1	12.8	1.18	1.97	37.7	3.1	1.85	22.1
	Mosaic L	241.2	12.8	1.10	2.20	26.8	28.7	2.16	13.9
P. C. L. + 500 N and 1000 P	Healthy	464.8	14.5	1.25	1.25	37.1		1.46	27.1
	Mosaic C	335.0	14.8	0.94	1.41	26.8	26.4	2.64	11.0
	Mosaic L	256.3	10.5	1.10	1.25	12.2	63.2	2.65	5.5
P. C. L. + 250 N and 4000 P	Healthy	360.4	14.7	0.94	1.26	38.4		1.39	29.2
	Mosaic C	274.1	11.8	0.94	0.94	32.9	14.3	1.63	21.4
P. C. L. + 250 N and 1000 P	Healthy	331.8	13.7	1.10	1.70	38.4		1.02	40.4
	Mosaic C	238.8	12.5	1.10	1.10	29.6	22.8	1.97	16.2
	Mosaic L	184.6	11.8	1.10	1.40	26.8	29.0	1.88	15.6

[1] Percentage based on dry weight.

[2] Poor clay loam plus 500 lbs. NH_4NO_3 and 4,000 lbs. 16 per cent acid phosphate per acre.

the mosaic plants. Even on the fresh weight basis, however, there was no evidence of a decrease in total nitrogen due to the disease.

In general, the more severe type of mosaic (L) caused the greater internal changes in the host. For example, the L type caused a reduction in total fresh weight of 44 to 56 per cent, the less severe C type, 5 to 36 per cent; the L type reduced the carbohydrate content 28 to 63 per cent, the C type 3 to 44 per cent.

In table 2 are shown the results of a bulk analysis of ten mosaic plants as compared with ten healthy ones of the Greater Baltimore variety grown in a greenhouse bench in June, 1925, and analyzed 23 days after inoculation. Seven of the inoculated plants showed only typical mosaic, and three the streak type. These plants were younger than those previously analyzed.

TABLE 2.—*Effect of mosaic on composition of young tomato plants*

Analytical tests	Healthy	Mosaic
Average fresh weight per plant	149.2 gm.	102.9 gm.
Dry matter	11.2 per cent	12.8 per cent
Reducing sugars	0.88 do	1.00 do
Non-reducing sugars	2.12 do	1.50 do
Polysaccharides	19.7 do	16.4 do
Total nitrogen	2.46 do	2.31 do
Carbohydrate-nitrogen ratio	9.23	8.18

The results in table 2 show that the mosaic plants were characterized by a reduction of 31 per cent in weight, 16.7 per cent in carbohydrate content, and 11 per cent. in the carbohydrate-nitrogen ratio. On the fresh weight basis there was no reduction in the nitrogen content.

TESTS WITH TYPICAL TOMATO MOSAIC

In the 1925 pot series, different soil conditions were provided by the addition of varying amounts of nitrogen and phosphorus to the poor clay loam, and of nitrogen, phosphorus, and potassium to Ottawa sand. Four pots of each type of soil were used. On October 29, 1925, a small plant of the Greater Baltimore variety (Indiana strain) was transplanted to each pot. On November 18, two of the plants in each series were inoculated with typical mosaic [type A of Fernow (10)], and the other two were left as uninoculated controls. On January 8, 51 days after inoculation, after the first fruits had set but before any had approached maturity, the two mosaic plants and two healthy plants from each series were taken for fresh weight determination and chemical analysis. The method of analysis varied only slightly from that previously outlined. The results are shown in tables 3 and 4.

TABLE 3.—*Effect of mosaic on growth and on carbohydrate and nitrogen content of tomato plants grown in poor clay loam with different fertilizer additions*

Fertilizer	Condition of plants	Average fresh weight in grams	Percentage[1] reducing sugars	Percentage[1] non-reducing sugars	Percentage[1] polysaccharides	Percentage reduction in total carbohydrate content	Percentage[1] total N	Carbohydrate-Nitrogen ratio
500 N +	Healthy	387	2.08	2.47	32.5		2.12	17.5
1700 P[2]	Mosaic	328	1.14	1.99	20.5	36.2	2.52	9.4
500 N +	Healthy	373	1.56	2.59	35.0		2.11	18.5
1200 P	Mosaic	330	0.94	1.96	21.0	38.9	2.31	10.3
500 N +	Healthy	311	1.25	1.88	28.0		2.16	14.4
700 P	Mosaic	306	1.35	1.66	25.0	10.0	2.44	11.4
500 N +	Healthy	180	1.45	1.55	22.0		2.63	9.5
200 P	Mosaic	192	1.00	1.50	19.0	14.0	2.28	9.4
125 N +	Healthy	208	1.39	1.74	32.0		1.68	20.9
1200 P	Mosaic	170	1.17	1.34	28.1	12.8	1.42	21.5
125 N +	Healthy	129	1.25	1.45	29.0		1.96	16.2
200 P	Mosaic	111	1.00	1.50	26.5	8.5	2.17	13.3

[1] Percentages based on dry weight.

[2] 500 lbs. NH_4NO_3 + 1,700 lbs. 20 per cent acid phosphate per acre.

TABLE 4.—*Effect of mosaic on growth and on carbohydrate and nitrogen content of tomato plants grown in Ottawa sand with different fertilizer additions*

Fertilizer	Condition of plants	Average fresh weight in grams	Percentage[1] reducing sugars	Percentage[1] non-reducing sugars	Percentage[1] polysaccharides	Percentage reduction in total carbohydrate content	Percentage[1] total N	Carbohydrate-Nitrogen ratio
500 N, 1500 P, 700 K, 400 Mg, 200 Fe[2]	Healthy	181	1.56	1.51	20.0		4.16	5.5
	Mosaic	134	0.78	1.52	19.0	7.6	3.59	6.0
500 N, 1500 P, 700 K	Healthy[3]	136	0.93	1.42	22.6		3.48	7.1
	Mosaic	160	1.10	1.19	21.5	4.6	3.55	6.7
100 N, 1500 P, 700 K. (Low N)	Healthy	55	1.11	1.97	36.7		2.06	19.3
	Mosaic	55	0.70	0.86	23.0	38.5	3.40	7.2
500 N, 75 P, 700 K. (Low P)	Healthy[3]	123	1.28	1.22	31.0		3.47	9.7
	Mosaic	25	0.75	1.45	26.7	13.7	3.95	7.3
500 N, 1500 P, 100 K. (Low K)	Healthy	94	0.78	1.22	35.0		4.56	8.1
	Mosaic	126	0.78	1.09	30.0	13.8	3.88	8.2
100 N, 75 P, 700 K. (Low N, P)	Healthy	37	0.90	1.30	36.3		1.83	21.0
	Mosaic	36	1.21	1.67	33.4	5.7	1.85	19.6
100 N, 1500 P, 100 K. (Low N, K)	Healthy	56	0.94	1.52	33.0		2.02	17.5
	Mosaic	46	0.88	1.62	25.0	22.4	2.26	12.1
500 N, 75 P, 100 K. (Low P, K)	Healthy	66	1.25	2.35	21.0		3.34	7.4
	Mosaic	75	0.85	1.65	20.5	6.5	4.20	5.5

[1] Percentage based on dry weight.

[2] 500 lbs. NH_4NO_3, 1500 lbs. $Ca_2(H_2PO_4)_2$, 700 lbs. K_2SO_4, 400 lbs. Mg_2SO_4, and 200 lbs. Fe_2SO_4 per acre. All pots received 200 lbs. $CaCO_3$ per acre.

[3] One plant used.

As in the 1924 series, there was no marked effect of different soil conditions upon the symptoms or severity of the disease, although these conditions did greatly affect the growth and composition of the plants. In general, the typical mosaic exerted in a somewhat milder degree the same effect upon the physiology of the host plant as did the double-virus type. In most cases the disease retarded growth as measured by fresh weight, and in all cases reduced the percentage content of carbohydrates. The nitrogen content was not consistently altered, and in most cases the carbohydrate-nitrogen ratio was lower in the mosaic plants.

DISCUSSION OF RESULTS

A reduced carbohydrate content and a normal nitrogen content in tomato mosaic are somewhat at variance with the phenomena found in similar diseases of certain other hosts. There was no marked decrease in total nitrogen as Jodidi, Kellogg, and True (11), and Jodidi, Moulton, and Markley (12) found in spinach mosaic, and hence no evidence that the denitrification theory advanced by Boncquet (2) and by Jodidi, Moulton, and Markley (12) will hold for the types of tomato mosaic studied. Likewise, the reduction of the carbohydrate content is the reverse of what happens in spinach mosaic as determined by True and Hawkins (21) and in potato leaf roll as determined quantitatively by Campbell (4), and lends no support to the theory that an abnormally high starch accumulation may be a characteristic of mosaic diseases in general.

In beet curly top, Bunzell (3) found little or no effect on the sugar or nitrogen content. In sugar cane mosaic, Lopez (16) found very little effect on the sugar content, but Asuncion (1) found 24 per cent less sugar in the diseased plants. Loew (15, p. 11) states that, in the case of tobacco, there is much less albumin in the mosaic leaves, and Woods (23, p. 13) states that the mosaic disease of tomato and tobacco is characterized by an accumulation of starch in the leaves, a view with which Tollenaar (20, p. 94) concurred.

W. P. Allyn found, in the course of work as yet unpublished (M. S. thesis, Purdue University, 1926), that starch constituted about 50 per cent of the polysaccharides in tomato plants and that there was a decrease in starch in the mosaic plants proportional to the decrease in polysaccharides. Furthermore, Allyn found that the mosaic diseases of cowpea and of soybean are also characterized by a reduction in the carbohydrate content.

Many of the earlier workers [Beijerinck, Koning (14, p. 69), Woods (22, p. 753)] believed that the mosaic disease of tobacco exerted an injurious effect upon the chloroplasts or the chlorophyll, and Dickson (5, p. 30) has shown that there is a decrease in number and a degeneration of the chloroplasts in the chlorotic areas accompanied by a decrease in carbohydrates.

lbs. $CaCO_3$ per acre.
[3] One plant used.

Elmer (9, p. 81) has shown by quantitative tests that the mosaic disease of tomato greatly altered the composition of the chlorophyll. Sorokin (17) and Eckerson (8) have demonstrated cytologically the disintegration of the chloroplasts in tomato mosaic. Consequently, with these various disturbances of the apparatus involved in carbohydrate synthesis, it is not surprising that a reduced carbohydrate content was found in the diseased plants.

SUMMARY

1. Different soil conditions, as afforded by pot tests with fertilizers, did not greatly influence the character or severity of either of the two types of tomato mosaic tested, the streak or double-virus type and typical tomato mosaic.

2. In nearly all cases the mosaic plants were characterized by a reduction in total weight and, under all conditions, by a reduction in total carbohydrate content as shown by chemical analysis. This reduction occurred mainly in the polysaccharides.

3. There was, in general, no reduction in the nitrogen content and, as a rule, the carbohydrate-nitrogen ratio was lower in the diseased plants.

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STUDIES ON THE NATURE OF THE VIRUS OF TOBACCO MOSAIC[1]

MAURICE MULVANIA

INTRODUCTION

It has now been 34 years since Iwanowski (15) showed that the active principle in the production of the mosaic disease of tobacco would pass through a fine earthen filter. This was the beginning of the study of the so-called filterable viruses which recently has assumed so much importance both in plant and animal pathology. The nature of the causal agent responsible for tobacco mosaic has been the chief point of interest in practically all studies of this disorder, so far as the purely scientific aspect of the subject is concerned. The effort devoted to such investigations has been very great, yet so far as exact knowledge of the intimate nature of the virus is concerned it can not be said that very notable progress has been made. To be sure, these efforts have resulted in the accumulation of a large mass of facts as to the response of the virus to environmental conditions. These facts, however, are not sufficiently enlightening to permit the allocation of the virus either with non-living or living bodies: there is little agreement on this latter point among workers most intimately acquainted with the subject. In this direction attempted enlightenment seems to have enhanced the obscurity it was expected to displace.

The applicability of certain experimental procedures to the question of the nature of viruses appears to have been overlooked in some cases, and not to have been employed to the extent of their possibilities in this connection in other instances. Use has been made of some of these methods in the studies here reported; and it is hoped that the objective results secured may aid in the ultimate solution of the perplexing problem as to what viruses really are.

Experiments were conducted in five independent aspects of the general subject dealt with. These phases of the problem may be listed as follows:

1. Effect of light rays on the virus of tobacco mosaic
2. Effect of heat on the virus of tobacco mosaic
3. Effect of animal inoculation on the virus of tobacco mosaic

[1] The following studies were made under the direction of Dr. James Johnson, of the University of Wisconsin, to whom I am greatly indebted for his assistance and sustained interest in the work.

4. Dialyzability of the virus of tobacco mosaic

5. Effect of bacteria on the virus of tobacco mosaic. Each of these headings is considered as a separate unit in the body of the paper.

The general literature concerned with tobacco mosaic has been repeatedly reviewed by others and therefore will not be considered here except as it bears directly on the problem under discussion.

EFFECT OF LIGHT RAYS ON THE VIRUS OF TOBACCO MOSAIC

Raciborski (23) found that exposure of plant juice which contained the active principle of mosaic to sunlight for one day did not destroy the virus. Four or five days exposure was necessary to render it harmless to healthy plants when they were inoculated with it. His exposures were made in

TABLE 1.—*The effect of sunlight on the virus of tobacco mosaic*

Time of exposure in hours	No. plants inoculated	No. plants infected	Per cent of infection
None	10	10	100
¼	10	10	100
1	10	10	100
2	10	10	100
3	10	10	100
6	10	10	100
12	10	8	80
16	10	5	50
25	10	2	20
36	10	1	10

shallow dishes. The results are rather indefinite, and, as they constitute the only published data on this subject, should be verified and extended.

A number of detailed methods of exposing bacteria to the action of light have been described and used. Among these the very simple procedure of saturating threads with a culture of the organisms and then exposing the threads to the light appears quite as satisfactory as any, and this method was selected for use in our experiments on the action of daylight on the mosaic virus.

Fine silk threads were placed in the expressed juice of diseased plants and allowed to remain for 24 hours. They were then suspended from a cardboard in a position where the sunlight would fall directly upon them during the entire day. These threads were removed from the light according to a time schedule fixed upon, and placed in 1 cc. of water for 24 hours. The water in which the threads had thus been soaked was used as inoculum for young tobacco plants. No effort was made to prevent bacterial con-

tamination of the solutions, since the presence of such organisms could have no bearing on the development of the mosaic in the inoculated plants. The number of infections which resulted from the inoculations was taken as an index of the destructive action of light on the virus. Throughout the entire series of experiments this has been the criterion as to whether the virus had been affected by its subjection to the various treatments. The results secured by the sunlight experiments are shown in table 1.

Exposures of the virus to the action of ultra-violet rays were made in petri dishes. Berkfeld F candle filtrates from diseased tobacco plants were placed in the dishes to a depth of $\frac{1}{8}$ inch. The uncovered dishes were then put immediately under a Cooper-Hewett mercury vapor lamp at a distance

TABLE 2.—*The effect of ultra-violet light on the virus of tobacco mosaic*

Time of exposure in hours	No. plants inoculated	No. plants infected	Per cent of infection
Series 1			
None	10	10	100
$\frac{1}{8}$	10	5	50
$\frac{1}{2}$	10	6	60
1	10	0	0
2	10	0	0
4	10	0	0
Series 2			
None	10	10	100
$\frac{1}{2}$	10	6	60
$\frac{3}{4}$	10	2	20
1	10	0	0

of six inches from the source of the rays. Observations of the temperature of the exposed solution showed that it did not at any time exceed 40° C. This temperature was found by other tests to be quite harmless to the virus even when maintained for many days. The time schedule and findings are indicated in table 2.

A rather small number of exposures of the virus to X-rays of light were made; and for the sake of suggestion are included here in table 3. It is not felt, however, that enough work was done in this connection to justify generalizations.

Considering the very great amount of effort which has been made to relate bacteria and the virus of tobacco mosaic, it is somewhat surprising

that a more careful comparison of the effect of light on the two should not have been made.

The foregoing results show clearly that the mosaic virus is strongly resistant to the influence of the entire solar beam of light, as well as to the portion at the extreme end of vibration frequency. This is, of course, in direct contrast to the action of light on bacteria. Bacteria in general are destroyed in from 1 to 2 hours in sunlight and in from 1 to 5 minutes by ultra-violet rays. Enzymes, on the other hand, are very resistant to

TABLE 3.—*The effect of X-rays on the virus of tobacco mosaic*

Time of exposure in minutes	No. plants inoculated	No. plants infected	Per cent of infection
None	10	10	100
5	10	8	80
10	10	10	100
20	10	10	100
30	10	9	100

the action of light, in some cases appearing not to be completely inactivated by indefinite exposure, although reduced in their zymogenic power. Thus it appears that in this particular the virus behaves like an enzyme and not like a bacterium.

EFFECT OF HEAT ON THE VIRUS OF TOBACCO MOSAIC

Considerable attention has been given to the action of heat on the virus of tobacco mosaic. This relation has been studied from two points of view: first, the temperature required to inactivate or kill the virus; and, second, the influence of heat on the progress of the disease in the host plant.

The determination of the thermal death-point of the virus under any given set of conditions is, of course, relatively easy, and this was ascertained with some degree of certainty in early studies of mosaic. Mayer (20) claimed that heating the virus to 60° C. did not destroy it; at 65°–75° C. it was attenuated, and heating to 80° C. killed the infective agent. Beijerinck (5) states that heating to the boiling point destroyed the disease-producing power of the virus. Chapman (7) found that heating at 80° C. for a short time killed the virus. Raciborski (23) showed that heating the sap from a diseased plant for 5 minutes at 62° C. did not cause it to lose its infective power. When heated to 100° C. for 15 minutes, however, the infective quality was lost.

As will be noted, three of these reports give no time limit to the heat application. In Raciborski's findings the time control is definite; yet the interval between 62° C. for 5 minutes and 100° C. for 15 minutes is very

wide and leaves a considerable range of possibilities. The second aspect of heat relation of the virus, namely, the effect of temperature on the progress of the disorder in the host, has been carefully studied by Johnson (16). He found that the symptoms of the disease in the plant could be completely masked by subjecting it to a rather radical change of temperature.

TABLE 4.—*The effect of long-time exposure of the virus of tobacco mosaic to different temperatures*

Temperature °C.	Time of exposure in days	No. plants inoculated	No. plants infected	Per cent of infection
15	10	5	4	80
25	10	5	5	100
35	10	5	4	80
45	10	5	5	100
15	16	5	5	100
25	16	5	5	100
35	16	5	4	80
45	16	5	5	100
55	16	10	8	80
65	12	10	9	90
75	4	10	8	80
75	8	10	8	80
80	1	5	5	100
80	2	5	5	100
80	3	5	3	60

These symptoms would reappear, however, if the plant was returned to the original heat environment. This phenomenon will be discussed later.

Experiments were conducted to determine the effect of long exposure of the mosaic virus to temperatures ranging from 15° C. to 84° C., the part that moisture plays in the destructive action of heat, and the thermal death-point of the virus when heat is applied for 10 minutes. In the tests made to determine the influence of long subjection of the virus to heat, 15° C. was selected as a starting point and 10 days as the time limit. With the time factor constant, the temperature was shifted upward until 45° C. had been reached. After preliminary results had been secured, both the temperature and time factors were varied according to the dictates of judgment as to what outcome might be looked for. In these experiments the virus was contained in freshly expressed juice of diseased plants. In all cases the infectiousness of the juice was ascertained at the beginning of the tests. Temperature control was secured in some of the experiments by the use of an electrical incubator, in others in an electrically heated and stirred water

TABLE 5.—*The comparative effect of heat on the virus of tobacco mosaic in solution and dry state*

Condition of virus	Temperature °C.	Time of exposure in hours	No. plants inoculated	No. plants infected	Per cent of infection
Solution	82	2	10	10	100
do	82	4	10	10	100
do	82	6	10	10	100
Dry	82	2	10	10	100
do	82	4	10	10	100
do	82	6	10	10	100
Solution	82	10	10	9	90
do	82	12	10	4	40
do	82	24	10	5	50
Dry	82	10	10	9	90
do	82	12	10	4	40
do	82	24	10	1	10
Solution	83	24	10	0	0
Dry	83	24	10	0	0
Solution	84	24	10	0	0
Dry	84	24	10	0	0

bath. In both methods the control was very satisfactory. Temperatures were always taken inside the test tube containing the virus.

In the experiments conducted to determine the relation of moisture to the destructive action of heat, equal amounts of powdered, diseased leaves were placed in test tubes. Half of the tubes were supplied with measured amounts of water while the other half were left dry during the period of heat application. The tubes were subjected to the heat in pairs, and after subjection the dry tubes were given the same amount of water as the others contained. In this way the dilution factor was made constant.

TABLE 6.—*The thermal death-point of virus of tobacco mosaic on 10-minute exposure basis*

Temperature °C.	Time of exposure in minutes	No. plants inoculated	No. plants infected	Per cent of infection
83	10	10	8	80
85	10	10	5	50
87	10	10	3	30
88	10	10	3	30
89	10	10	1	10
90	10	10	0	0
95	10	10	0	0

The 10-minute thermal death-point was determined on freshly expressed plant juice contained in test tubes, the only variable being the temperature.

Representative results of these experiments are given in tables 4, 5, and 6.

It is evident that the mosaic virus is very resistant to heat as compared with non-spore-bearing bacteria or bacterial toxins, withstanding temperatures of from 65° C. to 70° C. for many days without suffering definite reduction in its infective power. Even 80° C. for 3 days reduced it but 40 per cent in its disease-producing quality. Although there is considerable variation among enzymes in respect to their heat resistance, in general a higher temperature is required to inactivate them than is necessary to kill vegetative cells of bacteria. In this respect, then, the virus behaves less like bacteria than like enzymes.

The presence of moisture at the time of heat application appears to have no influence on the temperature required to inactivate the virus. This response is quite unlike that of bacteria, which uniformly yield more readily to heat in the presence of moisture. This latter reaction is held to be due to the fact that the coagulation point is lowered by moisture. If the virus is of bacterial nature it would seem that it should respond to the coagulation principle as other bacteria do.

The suppression of morbid symptoms in the host plant by a change of temperature may have at least two hypothetical explanations. In the first place the temperature may be sufficient to prevent growth and multiplication of the virus in the plant and thus check its harmful influence. Or the heat may influence the metabolism of the host plant in such a way as to overcome temporarily the action of the virus. It appears not impossible that the virus is a product of the plant's own growth. The interference with the growth of the plant might prevent its production of the harmful agent or virus. The progress of the disease is associated with the growing portions of the plant, and, if growth is seriously interfered with, the disease would in turn be checked. It is certain that a temperature sufficient to mask the disease symptoms is not high enough to destroy the virus when separated from the host.

EFFECT OF ANIMAL INOCULATION ON THE VIRUS OF TOBACCO MOSAIC

The literature appears to contain no references to the use of animal inoculation in the study of the mosaic virus. It is, of course, well known that non-pathogenic bacteria soon disappear from the blood of animals which have received them into their circulation. It is important to know what fate would overtake the virus if introduced into an animal's circulation.

The interrelation between plant and animal pathology perhaps has not been so strongly emphasized as could be desired; and the methods employed in one field have not been used to the limit of their usefulness in the other. Virus diseases seem to offer mutual ground for investigation as far as their causative agents are concerned. Although positive knowledge is still lacking as to whether viruses should all be placed in the same category, they are usually classed together. Such facts as have been secured indicate, however, that generalizations in this direction are largely unwarranted without fuller information.

Experiments were conducted to determine whether the mosaic virus could be recovered from the blood-stream of rabbits into which it had been injected. Chamberlain candle filtrates were used as inocula into the ear vein of rabbits which weighed 1500 to 1700 grams. Beginning with a dose of 1 cc., gradual increases were made until 3 cc. doses were given. At the end of a specified time after injection, samples of blood were drawn from the vein of the ear opposite to that which had received the injection. These samples were tested for the presence of infective virus. Results of these tests are presented in table 7. Recovery of the virus was not possible at any time at which samples were taken from the animals' blood.

TABLE 7.—*The effect on the virus of tobacco mosaic of injecting it into the blood-stream of rabbits*

Injection filtrate cc.	Time in minutes in the animal	No. plants inoculated	No. plants infected
1	20	10	0
1	30	10	0
2	20	10	0
2	30	10	0
2½	20	10	0
2½	30	10	0
3	20	10	0
3	30	10	0

In seeking an explanation of this disappearance of the virus from the circulation of the rabbit, tests were made to determine whether the destructive action was due to the blood. Blood was drawn from the ear vein of normal animals and immediately mixed with equal amounts of filtrates from infected plants. Plant inoculations were made from this mixture according to the schedule given in table 8. In other tests the blood was mixed with expressed infectious plant juice which had not been filtered (table 9).

It is shown that the fresh blood of a rabbit is capable of destroying the infective quality of the mosaic virus. According to the results of Freiburg

(10), when talc is mixed with plant solutions containing the virus, the latter is destroyed. This is held to be a case of selective adsorption. Duggar[2] has shown that the juice of pokeweed will act on the mosaic virus in a way similar to that of the action of talc. It may well be that, in the case of the destructive action of animal blood, we have encountered another example of selective adsorption.

Whether it is the serum or the cellular elements of the blood which inactivates the mosaic virus cannot be stated at present. Results secured by a few inoculations with centrifugally separated serum and cells mixed with the virus were inconclusive.

TABLE 8.—*The effect of drawn rabbit blood on the virus of tobacco mosaic in filtrate*

Blood and filtrate	Time in hours in contact	No. plants inoculated	No. plants infected
Equal parts	½	10	10
Equal parts	6	10	7
Equal parts	12	10	0
Equal parts	24	10	0
Filtrate alone	—	10	8

TABLE 9.—*The effect of drawn rabbit blood on the virus of tobacco mosaic in raw juice*

Blood and raw juice	Time in hours in contact	No. plants inoculated	No. plants infected
Equal parts	2½	10	10
Equal parts	24	10	0
4 to 1	2	10	7
4 to 1	24	10	5
Juice alone	—	10	10

DIALYZABILITY OF THE VIRUS OF TOBACCO MOSAIC

Since the original studies of Iwanowski (15) showed that the causal agent of tobacco mosaic would pass through fine earthen filter candles, numerous filtration processes have been applied to its study. The passage through such filters has been regarded as merely mechanical, depending on the relative size of the bodies to be filtered and the porosity of the candle, and has not been looked upon as a means of separating different kinds of suspensoids from each other on the basis of their particular colloidal properties. For the latter purpose resort has usually been made to various kinds of dialyzing membranes such as parchment paper, collodion sacs, and

[2] Unpublished results.

animal membranes. In connection with the study of the mosaic virus only two references to the use of such membranes are to be found in published form. Hunger (14) makes the mere statement that the virus will pass through parchment. Duggar and Karrer (8) introduced collodion sacs into a series of studies on the size of the particles of the mosaic virus. These proved unsuccessful, however, and were given a single trial and discarded. It would seem possible at least to differentiate between the simple colloids and any organized cellular body. On the basis of the colloidal chemists' conception, proteins, as well as other colloids, consist of micellae or aggregates of molecules. The larger of such aggregates must be broken up in advance of passage through dialyzing membranes. Formed proteins

TABLE 10.—*The dialyzability of the virus of tobacco mosaic through different grades of collodion sacs*

Grade of sac: percentage of alcohol	No. plants inoculated	No. plants infected	Per cent of infection
20	10	9	90
30	15	8	53+
40	15	7	46+
45	10	0	0
50	15	0	0
60	10	0	0
70	10	0	0
Non-dialyzed juice	10	9	90

are thought to consist of relatively large groups of molecules, and are practically undialyzable. Our present notion of a living entity being that of complex protoplasm carrying a very considerable amount of proteins would lead to the conclusion that such entities could not possibly go through a membrane which would not allow their components to pass. It would require that such bodies be disrupted, and their protein constituents themselves would have to be broken into minor groups of molecules before even they could dialyze. Now we can not think of a cell sustaining such disintegration and retaining its integrity as a cell. Conversely, if a body should be found to have passed such dialyzing membranes as mentioned and still possess its original properties, it could not be regarded as a living protoplasmic unit.

According to the findings of Eggerth (9) and others, collodion sacs of different dialyzing properties may be made by varying the ratio of alcohol to ether as a solvent for the collodion. The higher the relative percentage of alcohol in the mixture, the easier will be the dialytic transfer of sub-

TABLE 11.—*Collected results of dialysis experiments on tobacco mosaic virus with different grades of collodion sacs*

Grade of sac: percentage of alcohol	No. plants inoculated	No. plants infected	Per cent of infection
20	30	20	66.6
25	20	15	75
30	50	8	16
35	40	9	22.5
40	40	10	25
45	70	3	42
50	110	5 (4 from 1 sac)	4.5
50 acid	80	25	31
50 salt	20	0	0
50 NaOH	20	0	0
55 acid	40	14	23+
55	10	0	0

stances. Thus, a sac made from collodion dissolved in 75 per cent alcohol and 25 per cent ether solution will permit passage of a substance which would be entirely blocked by a sac made from 25 per cent alcohol and 75 per cent ether solution.

The interest we had in finding a sac which would permit the passage of the virus of mosaic led to the making of a graded series from 20 to 70 per cent alcohol solutions, ascending by intervals of 5 per cent.

The manipulations followed in making the collodion sacs were essentially those described by Gates (11) in a recent publication. Eight per cent of collodion is dissolved in the desired mixture of completely dehydrated alcohol and ether. The sacs are moulded in short test tubes, and a

TABLE 12.—*The influence of chemicals on the dialyzability of virus of tobacco mosaic through different grades of collodion sacs*

Grade of sac: percentage alcohol	Chemicals used	No. plants inoculated	No. plants infected	Per cent of infection
50	HCl, 2 per cent, N/20	30	7	23+
50	NaCl, 1 per cent	20	0	0
50	No chemicals	10	0	0
55	HCl, 1 per cent, N/20	40	14	35
55	NaOH, 1 per cent, N/20	30	0	0
55	Glucose, 1 per cent	20	1	5
55	No chemicals	20	0	0
Non-dialyzed juice		20	20	100

glass tube is inserted inside the sac within the test tube. The sac is then removed with the glass tube to which it adheres loosely. The tube is withdrawn from the bottom of the sac about one inch. After the sac in contact with the tube dries somewhat it will adhere tightly, and the lower portion will hang suspended below the glass tube.

In use, the glass tube with its sac is supported above a vessel containing the expressed juice from a plant known to be affected with mosaic. The sac is then partly filled with distilled water and lowered into the juice so that the free end is submerged. In this position it remains a specified length of time, when the contents of the sac are taken out with a pipette. The solution thus secured, consisting of the distilled water and whatever substance may have passed through the membrane, is used to inoculate young tobacco plants.

TABLE 13.—*The influence of electrical current on the dialyzability of virus of tobacco mosaic through collodion sacs*

Grade of sac: percentage alcohol	Direction of current flow	No. plants inoculated	No. plants infected
50	From juice to distilled H_2O	20	0
50	From distilled H_2O to juice	20	0

Tables 10–13 are representative of what was found in a very long series of experiments. It is observed that the mosaic virus will pass with a fair degree of certainty through sacs made from a solution containing from 20 to 45 per cent of alcohol as a solvent for the collodion. Sacs having a higher percentage of alcohol than 45 almost never allowed the virus to pass unless the juice carrying it had its pH value changed to the acid side. In this latter respect the virus behaves as a typical colloid, as it is known that a change of reaction slightly to the acid or alkaline side of the isoelectrical point will encourage colloids to pass such membranes. The addition of alkaline solutions to the juice containing the virus did not induce a passage of the latter through sacs impervious to it in the untreated juice. This is no doubt due to the fact that the juice is normally on the alkaline side of the isoelectrical point.

The addition of salt and sugar to the solution containing the virus did not change the dialyzing property of the virus. The results were likewise negative when an electrical current was passed across the membrane which separated the plant solution and virus from distilled water. The current was passed in both directions, that is, from the juice to the water and from the water to the juice. Hydrophylic organic colloids, according to Bechhold (4, p. 77), do not pass in any definite direction in an electrical field.

They acquire a definite polarity relation only on the addition to the solution of an electrolyte. If the virus in the plant juice acts as a colloid, we should not expect the passage of the current to change the property of movement through the sac if no electrolyte had been supplied. The fact, observed here, that the current does not increase the movement of the virus through the membrane points to a similarity between it and colloids.

EFFECT OF BACTERIA ON THE VIRUS OF TOBACCO MOSAIC

The fate of the mosaic virus in the soil is of very considerable economic importance and has received much attention. The reported results are not

TABLE 14.—*The influence of bacterial growth on the tobacco mosaic virus when cultured in equal parts of bouillon and filtrate*

Organism	Cultivation temp. °C.	No. plants inoculated	No. plants infected	Per cent of infection
B. subtilis	37	5	2	40
B. coli	37	5	1	20
B. suipestifer	37	10	2	20
B. prodigiosus	37	10	1	10
B. hartlibii	37	10	5	50
B. proteus vulgaris	37	10	1	10
B. anthracoides	37	10	3	30
B. ruber balticus	20	20	0	0
Sarcinia lutea	20	20	3	15
B. aerogenes	20	20	2	10
Cellulose fermenter	65	40	1	25
No bacteria	20	35	21	60
Check plants 30		0	0	0

concordant, yet on the whole they give one the impression that the virus is soon lost when introduced into field soils, or moist soils. Beijerinck (5) claimed that it was not destroyed by remaining in the soil over winter. Koning (18) could not produce the disease in healthy plants by inoculating them with soil solutions from fields where diseased plants had grown. Allard (1) secured results which indicated that the virus is soon lost or destroyed in field soil. In solutions of plant juice the virus is known to withstand the process of decomposition of the juice and remain infectious.

In the soil, of course, the virus is subjected to the action of a very complex biological condition, bacterial, enzymatic, and protozoan. It is manifestly impossible to know anything concerning the relative influence of any one of these factors under such conditions. Experiments were undertaken to show the individual effect of certain bacterial species on the virus. In all of the experiments conducted in this connection equal parts of filtrates

TABLE 15.—*The destructive action of selected bacteria on the virus of tobacco mosaic*

Organism	No. of inoculations	No. of infections	Per cent of infection
Sarcinia lutea	40	17	42.0 +
B. ruber balticus	40	7	17.0 +
B. proteus vulgaris	40	3	7.5
B. aerogenes	40	4	10.0
Cellulose fermenting bacillus	40	1	2.5
Cheek filtrate and bouillon	40	30	75.0
Filtrate alone	40	31	77.0 +

of diseased plant juice and sterile bouillon were placed aseptically in test tubes. After the mixture was shown to be free from bacteria it was seeded with the organism to be tested. Sufficient time was allowed for the bacteria introduced to complete their growth in the medium. Plants were then inoculated with the culture. The results are shown in table 14.

These results show that all of the bacteria included in the foregoing experiments possess some power to inactivate the virus of mosaic. Some show very little capacity in this direction, as in the case of *Bacillus hartlibii,*

TABLE 16.—*The effect of heat on enzymes, bacteria, animal viruses, and the mosaic virus of tobacco in moist and dry conditions*

Enzymes, bacteria, toxins, and viruses	Destruction temp. in degrees C.		Authority
	Wet	Dry	
Sucrase	55–75	160	Kayser (17, p. 60)
Amylase	75–80	130	do
Trypsine	70	100	do
Lipase	60	.....	do
B. tuberculosis	60	100	Hiss and Zinsser (13)
S. cholera	52	.....	do
B. typhosus	60	.....	do
M. gonorrhea	62	.....	do
B. anthracis spores	100	140	do
Diphtheria toxin	60	.....	Oppenheimer (22)
Botulinus toxin	58	.....	do
Symptomatic anthrax toxin	50	.....	do
Horse anaema virus	58	.....	Buchanan (6)
Fowl plague virus	55–60	.....	do
Cattle plague virus	58–60	.....	do
Hog cholera virus	60–70	.....	do
Tobacco mosaic virus	88–90	88–90	do

TABLE 17.—*The effect of light on enzymes, bacteria, toxins, and the mosaic virus of tobacco*

Enzymes, bacteria, toxins, and viruses	Loss of activity in per cent	Time of exposure	Authority
Malt diastase	68	——	Hill (12, p. 97)
Saliva diastase	45	——	do
Plant diastase	8	——	do
B. tuberculosis	100	1 hr.	Hiss and Zinsser (13)
M. gonorrhea	100	5–10 min.	do
Diphtheria toxin	100	1 hr.	do
Anthrax toxin	0–5	indefinite	Oppenheimer (22)
Botulinus toxin	100	1 hr.	do
B. anthracis spores	100	1 hr.	Arloing (3)
Tobacco mosaic virus	90	36 hrs.	

while others completely destroy the infectiousness of the virus, as, for instance, *B. ruber balticus*. The organisms used were selected without reference to their biological qualities, and there seems to be no possibility of distinguishing the vigorously destructive group from the non-destructive group on the basis of any recorded physiological or morphological characteristics.

A more extensive study was given to four selected members of the group of bacteria which had shown greatest capacity for destroying the virus. The figures given in table 15 for 40 inoculations were compiled from quadruplicate cultures of each bacterium, ten plants being inoculated from each culture. Duplication was very good in all cultures with a given organism.

The results merely emphasize the fact, already alluded to, that certain bacteria in pure cultures have a decided ability to overcome the disease producing faculty of the virus of tobacco mosaic.

TABLE 18.—*The relation of moisture to the coagulation of albumin and destruction of mosaic virus of tobacco by heat*

Material	Percentage water	Coagulation temperature in degrees C.	Death-point in degrees C.	Authority
Egg albumin	100	65	——	Lewith (19)
do	25	74–80	——	do
do	18	80–90	——	do
do	6	145	——	do
Mosaic virus	100	——	88–90	
do	Air dry	——	88–90	

An analysis of tables 16–18 shows that there is considerable variation among the members of all the groups of bodies considered as to their response to heat and light. In regard to the influence of heat, the mosaic virus behaves more as an enzyme than as any of the other classes of bodies. The animal viruses are destroyed uniformly at a comparatively low temperature, while bacteria and toxins fall into an intermediate position between them and the ferments. The mosaic virus displays one characteristic not noted in any of the other substances, namely its indifference to the moisture content as affected by heat. As is seen, the coagulation temperature of albumin depends on its content of water; whereas the mosaic virus is destroyed by heat independently of moisture. This indicates that its inactivation is not due to coagulation. Both mosaic virus and enzymes are very resistant to the action of light. Bacteria on the other hand are relatively sensitive to light.

One is led to conclude from these considerations that the mosaic virus of tobacco is more nearly related to enzymes than to either bacteria, animal viruses, or toxins as far as this kind of evidence permits generalization.

THEORETICAL DISCUSSION

Granting the accuracy of observation and validity of conclusions set out above concerning the viability of the causal agent of tobacco mosaic, we are obliged to seek a non-vitalistic explanation of the diseased condition of the plants. In so doing at least two hypothetical possibilities present themselves. It is generally conceded that in some way the virus is multiplied. How then may such increase be accomplished if the active agent is not a living entity? First, it may be conceived as a product of the plant's own metabolic activity under the influence of some disturbing factor introduced into the plant body, namely the virus produced by other plants similarly affected. Thus we invoke the principle of auto-catalysis. Autocatalyses of a purely chemical nature are well known, and organic autocatalyses are also recorded. The enzymatic transformation which takes place in the endosperm of germinating grain, as in the wheat grain, is perhaps of this character. The parallel multiplication ratio of the bacteriophage and the bacteria which it lyses during the lytic phenomenon, supported by other evidence, is being regarded as an auto-catalysis by an increasing number of investigators. Certain manifestations during the progress of the mosaic disease lend themselves readily to such an explanation. Among these may be mentioned the incubation period, the apparent modification of the virus during its sojourn in different hosts, the correlation between the progress of the disease, and cell division in the meristem tissue and the fact that the plant does not recover and is not killed by the disorder.

A second hypothesis may be stated as follows. It would seem to be essential to the theory of evolution to assume that somewhere, somehow, living entities sprang from inert matter. We distinguish between living and non-living things by noting certain qualities in one that are not observable in the other. In general these qualities are reproduction, nutrition, adjustment, and response to stimuli. These properties appear to be distinct qualities, that is, not dependent upon each other. These separate factors of life must have been acquired somewhere along the path of emergence from inert to living conditions. There is nothing in the logic of the case to prevent the assumption that these qualities may have been acquired one by one rather than simultaneously. There is nothing, then, to preclude our thinking that there may still be bodies, or aggregates of molecules, which are possessed of any one or more of these factors, situated along the way of evolution, bodies so to speak between the living and the dead as life is now conceived. Such bodies might well be able to reproduce without capacity for adjustment, response, or possibly nutrition.

The mosaic virus might be possessed of two of these qualities, namely reproduction and nutrition, or even that of reproduction alone, increase in quantity being some form of additive process incited by the host plant. The plant might thus add molecules to the initial foreign group up to the point of reproduction, the one function of life inherent in the initial foreign group of molecules. Now it is possible to see how this continuous addition of certain molecules to the foreign body could disturb the normal metabolism of the host plant. Molecules thus being taken out of their normal course would leave the plant without its due quota and thus prevent the maintenance of the nutritive balance normal to the growing plant. Or if, in addition to the quality of reproduction, the virus group of molecules should have that of nutrition as well, it might be seizing upon particular plant molecules appropriating them to itself. In either case the effect upon the host plant would be the same. Such a view, while entirely hypothetical, seems not to be outside the realm of possibility.

Mrowka (21) and Andriewsky (2), working on chicken pest independently and with different methods, came to the conclusion that the virus is a globulin. The same conclusion was reached by Sangiorgi (24). It is not impossible that the mosaic virus of tobacco may be of similar nature, that is, a protein of very simple kind, having the characteristics of an enzyme.

SUMMARY

1. Experiments have been conducted to show the influence of light on the virus of tobacco mosaic. The results show that it is very resistant to the action of light. Exposure of the virus to direct sunlight for 36

hours destroyed 90 per cent of its infectiousness. Ultra-violet light inactivated the virus in one hour. X-rays do not appear to injure it.

2. Exposure of the virus of tobacco mosaic to 80° C. for as long as 20 days did not completely destroy it. The thermal death-point of the virus on the 10-minute exposure basis is between 88° and 90° C. No relation between the moisture content of the material carrying the virus and the killing temperature could be detected.

3. It was not possible to recover the virus of tobacco mosaic from the bloodstream of a rabbit, into which it had been injected, at any time between 3 and 30 minutes. The injection caused no detectable reaction on the part of the animal. Drawn blood of the rabbit, when mixed with plant juice containing the virus, inactivated the latter. Results with cow and horse blood are less definite.

4. The virus of tobacco mosaic has been found to pass through certain grades of collodion sacs and not through others. A slight change of reaction of the juice containing the virus, to the acid side of the isoelectrical point, induced the passage of the virus through sacs impervious to normal juice. The passage of an electrical current across the sac does not cause the virus to dialyze. Sugar, salt, and alkalies are equally ineffective in this direction. These results lead to the conclusion that the virus of tobacco mosaic cannot be as complex a body as a biological cell, therefore not a living entity in the present accepted sense.

5. Certain bacteria have been found to be very destructive to the virus of tobacco mosaic when cultivated in a solution containing it. Other species are but slightly, if at all, harmful to it. Apparently there is no biological characteristic by means of which the two groups of bacteria can be separated. Therefore no suggestion can be made as to how the inactivating effect on the virus is produced.

6. The evidence gained by these experiments seems to point to the virus of tobacco mosaic as a non-living thing, possibly a very simple colloid, possibly also of protein nature and having enzymatic characteristics.

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ATTEMPTS TO CULTIVATE THE TOBACCO MOSAIC VIRUS

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Olitsky[1] has obtained evidence that the virus which causes the mosaic disease of tobacco and tomato can be cultivated *in vitro*. The virus, when transferred in dilutions to a suitable culture medium, which consists of the clear filtrate of normal tomato tissue, increases in potency after an incubation period at 30° C. The results of his experiments seem to justify the conclusions made. Apparently there was an increase in the causative agent.

Mulvania[2] carefully repeated the work. Tobacco plants inoculated with incubated tomato mosaic virus failed to show an increase in infection over that caused by the unincubated material. Furthermore he showed that diluted virus suspended in distilled water and incubated gave as much infection as the incubated material in the culture medium. Mulvania concludes that the mosaic virus of tobacco and tomato can not be cultured according to the method outlined by Olitsky.

The findings of these two workers suggest the need of further work on the subject. The writer has repeated the work, using tobacco plants throughout the experiment. Olitsky used tomato plants; Mulvania used tomato and tobacco. Since the disease of both plants is caused by the same virus, either system or a combination of both may be used.

The experiment was carried out under greenhouse conditions. The tobacco plants were grown from seeds and potted in 6-inch pots. Ten plants were used for each test.

The experiment was carried out in two series. The virus for the first series was obtained from dried diseased tobacco leaves sent by Prof. A. V. Osmun of the Massachusetts Agricultural College 18 months previously. The virus for the second series was secured from diseased tobacco plants resulting from the first inoculations.

In both cases the virus was secured by mincing and crushing the mosaic tissues with distilled water in a mortar. The resulting fluid was then passed twice through Berkefeld filters of V porosity. The clear filtrate was tubed and incubated for 10 days at room temperature in order to test its sterility.

The culture medium of Olitsky's was obtained by mincing, crushing and filtering normal tobacco tissues as above. The clear solution gave,

[1] Olitsky, P. K. Experiments on the cultivation of the active agent in mosaic disease in tobacco and tomato plants. Jour. Exp. Med. 41: 129–136. 1925.

[2] Mulvania, M. Cultivation of the virus of tobacco mosaic by the method of Olitsky. Science 62: 37. 1925.

using the hydrogen electrode, a pH of 5.9. The solution was tubed in 10 c.c. lots and allowed to remain in the incubator for 10 days at 30° C. before inoculating with the dilutions of the mosaic virus. The tubes remained clear after this incubation period.

After this preliminary sterility test, the culture tubes were inoculated with dilutions of the virus solutions. The dilutions were made by transferring 0.1 c.c. of virus solution to 10.0 c.c. of the culture fluid. Dilutions of 1/100, 1/10,000 and 1/1,000,000 were obtained. Undiluted and diluted tubes were then incubated for 8 days at 30° C.

One set of 10 plants in each series was inoculated with the original virus solution after passing through the filters. The incubated material was inoculated into sets of 10 plants. The inoculations were made by injecting the material from the culture tubes into the axils of the leaves with sterile pasteur pipettes. Check lots of plants were placed among the inoculated sets. These were inoculated with sterilized distilled water.

Table 1 gives the number of the series, magnitude of the dilution, and the number of diseased plants.

TABLE 1.—*Results of inoculating tobacco plants with mosaic virus of varying dilutions*

Dilutions	Incubation for 8 days	Series 1 Mosaic virus obtained from dried leaves stored 18 months. Ten plants in each set.	Series 2 Mosaic virus from a diseased plant from Series 1. Ten plants used in each.
		No. diseased plants	No. diseased plants
Undiluted		5	10
do	30° C.	6	10
1/100	do	2	7
1/10,000	do	0	3
1/1,000,000 A	do	0	1
1/1,000,000 B	do	1	0
1/1,000,000 C	do	0	0
1/1,000,000 D	do	0	0
1/100 of A	do	0	....
1/10,000 of A	do	0	....
1/1,000,000 of A	do	0	....
Distilled water (check) A		0	0
Distilled water (check) B		0	0
Distilled water (check) C		0	....

CONCLUSIONS

Olitsky's findings have not been verified during the course of the experiment on tobacco mosaic. Diluted tobacco mosaic virus did not increase its

potency when incubated in the culture medium described by Olitsky. According to the findings of Olitsky one would expect the diluted cultures to give, after the incubation period, as much infection as the original undiluted virus solution. Especially should this be so with the lower dilutions. The results of this experiment support the findings of Mulvania. There is a gradual decrease in the number of infections as the virus is diluted, and in only two cases out of eight did the 1/1,000,000 dilutions give any infection. No infections occurred when the 1/1,000,000 incubated dilutions were further diluted.

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STUDIES ON THE SPOT DISEASE OF CAULIFLOWER; A USE OF SERUM DIAGNOSIS

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This disease, first described by Lucia McCulloch (3) and later by Erwin F. Smith (4), appears to be of growing economic importance throughout the cauliflower plantings of the San Francisco Bay Region in California. In the fields and in the local markets a great many of the heads are affected. This is especially so at the height of the season, during February and March. At this time nearly all of the heads are spotted.

The disease is easily recognized by the small brown or dark brown spots that appear on the inflorescence or edible part of the plant. These spots vary in size, sometimes reaching several millimeters in diameter; they are at first firm but later become soft. As the disease progresses, invasion by saprophytic or secondary organisms occurs, and the decay becomes characterized by a very offensive odor. Sometimes the heads are slightly spotted, but during severe attacks the whole inflorescence becomes covered with these brown, firm areas. The spots are confined to the epidermal and adjacent layers of parenchyma cells, and very seldom are they found affecting the deeper tissues.

The observations of the writer indicate that the disease is commonly one of over-mature cauliflower heads. Occasionally it occurs after injuries. In the field one finds the spots on heads that have been mature for a period before picking. Since size is a market factor of some importance, the tendency is for the grower to try to get as large a head as possible. For this reason many of the heads are allowed to remain in the field longer than necessary, and consequently become spotted.

Sharp frosts, such as California experiences during the months of January, February and March, indirectly cause considerable damage to the cauliflower inflorescence. There seems to be no direct frost damage as is usually evidenced by blackened tissue, but nevertheless the brown spots increase considerably in numbers after these sharp changes in temperature. There seems to be just enough injury to the epidermal cells of the fleshy inflorescence to allow the invasion of the organism causing the brown spots.

The writer has observed repeatedly, during the winters of 1922–23, 1923–24 and 1924–25, that, following these changes in temperature, considerable spotting of the cauliflower heads occurred in the fields. On the market stands the heads were often 100 per cent affected. During these

periods the infections were general, and seldom was one able to find a head of cauliflower free from spots.

The writer has repeatedly isolated the causal organism, *Bacterium maculicolum*, described by McCulloch. This organism can readily be isolated in pure cultures from the spots when they are small and firm. When the spots are soft, other organisms appear on the plates. The isolated organism, when sprayed on cauliflower heads under sterile laboratory conditions, readily reproduces the disease. The writer has repeatedly carried it through three generations on cauliflower heads.

The organism isolated by the writer agrees culturally and morphologically with that described by Miss McCulloch. The writer did not succeed in producing the black spots on young cauliflower seedlings as McCulloch was able to do. She noted that only recently isolated organisms were capable of producing these spots and attributed the non-production of lesions by older cultures to a loss of virulence due to age. The writer used both young and old cultures without success.

The organism is gram negative, stains readily with the ordinary dyes, and is non-acid fast. A short rod, occurring usually in pairs, sometimes as short chains. Motile by two to five polar flagellae (Plimmer and Paine method). Gelatin moderately liquified. Lactose, saccharose, maltose, dextrose, xylose, mannite, and glycerin not fermented. Acid not formed in above. Slight acid formation in glucose. Strictly aerobic (confined to the open arm of fermentation tube). Grows moderately on beef bouillon agar slants, profusely on Czapeks synthetic sugar media. Forms a yellow-green pigment in gelatin, beef bouillon and in solid media. No nitrite or indol formed. Grows readily at 37° C., also at 4° C. or ice box temperature. No spores are formed. Milk is peptonized. Agglutinins are readily incited in the serum of rabbits. A titre of 1/10,000 was obtained.

PERSISTENCE IN SOIL

Since many of the cauliflower heads are infected in the field, it was thought that the organism causing the brown spot might be a soil-inhabiting species. To determine this, an experiment dealing with the occurrence and persistence of this bacterial pathogen in the soil was conducted.

The literature dealing with experiments of this type was found to be rather unsatisfactory. The persistence of pathogenic bacterial organisms in the soil has often been dealt with in a casual way, and many writers have been content to designate the organisms that they were working with as overwintering in the soil without any experimental basis for their conclusions. That it is not easy to study pathogenic bacteria in the soil is obvious to many plant pathologists. The extreme difficulties of isolation of a spe-

cific form from among the various members of the bacterial flora often makes such a study almost impossible.

Fulton (1), working with *Pseudomonas citri*, the citrus scab organism, studied the decline of the organism in the soil. He recognized the difficulties of trying to plate out his organism from the soil, "because of the preponderance of more rapidly growing soil organisms, ordinary plating methods are inadequate for determining the abundance of *Ps. citri* in soil samples and recourse was had to inoculation of punctured mature grape-fruit leaves with . . . washings from the soil." Kotila and Coons (2), working on the method of overwintering in soil of the potato blackleg organism, *Bacillus atrosepticus*, also recognized the difficulties of the isolation of bacterial plant pathogenes from the soil. The difficulty experienced in endeavoring to pick up colonies of the blackleg organism from plates poured from soil samples is of course obvious. ". . . There is such a great number of soil organisms which develop on the plates, very similar to the blackleg organism, that the chances of picking up the right organism are very small." The above workers used the inoculation method for determining the persistence of their organisms in the soil. This method is open to criticism, in that successful inoculations are not always possible. It has been the writer's experience that successful inoculations are rare indeed in comparison with non-successful. The required relation between the parasite and the host may exist only at times and under certain conditions. It seems unsafe, therefore, to base conclusions on the presence of any parasitic organism on chance infections. Something more specific and, at the same time, easy of manipulation should be used in such studies on the persistence of organisms in the soil.

The writer, taking advantage of the high specificity of antibodies, as used by medical bacteriologists, has brought into use the agglutinating antiserum in his present study. High titre antiserums were produced by injecting into rabbits the appropriate antigen, namely, the organism under study. Identification of the isolated organism was made by agglutinating at high dilutions of the specific antiserum. In this manner the identification became easy and at the same time absolute.

The method of producing the agglutinins was as follows: 24-hour-old cultures growing on Bacto-peptone beef bouillon agar slants (pH 6.8) were suspended in 10 c.c. of physiological salt solution (0.85 per cent). The living organisms were injected intravenously into rabbits weighing about 2,500 grams as follows: on the first day 1.0 c.c. of the above saline suspension, 1.0 c.c. on the second day, and 1.5 c.c. on the third. Seven days after the first injection the series of three were then repeated with doses of 2.0 c.c. each day. Sixteen days after the first injection the animals were bled

from the heart. An excellent titre was obtained by this method. Phenolized cultures did not prove potent. The living suspensions proved to be slightly toxic for the rabbits. A little care must be taken during the first period of injections. The above method is one that is used in the medical bacteriological laboratory at the University of California.

The present work consisted of examination of sterilized and unsterilized flasked soil, inoculated with a broth suspension of *B. maculicolum*. The experiment was carried out under laboratory conditions.

METHODS OF EXPERIMENTATION

Erlenmeyer flasks, capacity 250 c.c., in which 150 grams of ordinary campus soil was placed, were used for the experiment. The campus soil is a heavy clay loam in which are dispersed small and large pieces of native rock. This soil had an initial reaction of pH 6.5 as determined by the hydrogen electrode.

Three sets of 24 flasks of soil were used. One set was sterilized for 4 hours under pressure in the autoclave and inoculated with a suspension of *B. maculicolum* growing in beef bouillon broth. Another set was left unsterilized but was also treated with the suspension of organisms. Enough of the suspension was added in both cases to bring the moisture content of the soils to optimum. The last set was sterilized as above but not treated with the suspension. Distilled water was added to these instead of the suspension. This set was used for the checks. All of the flasks were then placed away in a laboratory cupboard for further examination. The flasks were not molested until used for the experiment. As each flask was opened it was discarded.

The isolations were made by placing a few grams of the flasked soil in a tube of sterile beef broth and incubating at room temperature until turbidity developed. Proper dilutions were then made of the culture, using Czapek's synthetic sucrose agar pH 6.6, which proved to be a very suitable substratum for the isolation of this organism, since it grows luxuriantly and gives a greenish pigment in the medium. Characteristic colonies were then selected and isolations made from these on nutrient beef agar slants. The identification was then made by use of the agglutinating anti-serum of rabbits.

Isolations were made each week, for the first four weeks, then every two weeks for sixteen weeks, then every four weeks for the remainder of the year. The experiment was begun September 17, 1924, and was continued until September 17, 1925.

The natural field conditions were not duplicated in the laboratory experiment. The temperature of the laboratory does not change very much

throughout the year. The moisture conditions under which the experiment was conducted obviously show no analogy with those met with in the field. The fluctuating environmental conditions, which no doubt play a great part in the persistence of any organism in the soil, were lacking in this experiment. The competitive influence of the other members of the soil flora may also play a part in this persistence. This competitive factor was encouraged to some extent in the unsterilized flasks. That the competitive factor was not wholly duplicated is recognized by the writer since certain types of the soil flora which might be present under normal conditions were absent.

The moisture content of the soil decreased slowly throughout the experiment. After two months the flasked soil was dry. At the end of the experiment and, in fact, during the last six months, the soil was powdery dry. The writer feels that, under these moisture conditions, the organisms were placed under quite a severe test.

The problem of identification was made simple by the agglutinating antiserum. Since the question of specificity might enter into the criticisms of this paper, the writer tested other organisms that were found growing on

TABLE 1.—*The growth*[a] *of Bacterium maculicolum and other organisms in sterilized and unsterilized soil. Experiment in progress from Nov. 17, 1924, to Nov. 17, 1925*

Time in weeks	Check (sterilized soil)		Inoculated (sterilized soil)		Inoculated (unsterilized soil)	
	B. maculicolum	Other organisms	*B. maculicolum*	Other organisms	*B. maculicolum*	Other organisms
1	–	×	×	–	×	×
2	–	–	×	×	×	×
3	–	×, f	×	–	–	×
4	–	–	×	–	×	×
5	–	–	×	–	×	×, f
7	–	×	×	×	lost	
9	–	–	×	–	×	×
11	–	–	×	–	×	×
15	–	×	×	×	–	×
19	–	–	×	–	×	×, f
23	–	–	×	–	×	×
27	–	–	×	–	×	×, f
35	–	×, f	×	–	×	×
40	–	–	×	–	×	×, f
44	–	–	×	–	×	×
48	–	–	×	–	–	×
52	–	–	×	–	×	×

[a] –, no growth; ×, growth; f, fungous growth.

the plates obtained from the unsterilized series. A few animal pathogenes, *Bacillus coli, Bacillus typhosus* and *Staphylococcus aureus,* as well as a few plant pathogenes, *Bacillus carotovorus* and two types of *Pseudomonas cerasus,* were also tested with the antiserum. No cross agglutination was observed. Furthermore the agglutinating antiserum was used in 1/1000 dilutions, a fact which obviates any error that might creep in from this direction.

Transfers were made from the characteristic colonies, found growing on the plates resulting from each of the inoculated flasks of soil, sterilized and unsterilized, on to nutrient beef agar slants. Transfers were also made from colonies developed on the plates from the check flasks, which proved to be unsterile. Many of the transfers represented colonies that resembled those of the organism inoculated into the soil. As stated before, these transfers were used to check the group relations if such existed. None of these were agglutinated by the specific antiserum in any dilution.

RESULTS

Table 1 shows the occurrence of *B. maculicolum* in the laboratory soil.

The data given in the table show how readily *B. maculicolum* can persist in soil under the conditions of the experiment. This bacterium appears to be well able to compete with the other members of the soil flora. In no case in this experiment was the organism found normally in non-inoculated soil. This was shown in the flasks which were sterilized but which were found later to harbor a flora. Apparently four hours' sterilization at 115° C. under pressure is not sufficient for flasked soils.

NATURAL OCCURRENCE OF BACTERIUM MACULICOLUM IN SOIL

The writer had occasion during the course of another experiment to accumulate cultures from various soils throughout the northern part of California. These soils represented areas devoted to orchards of deciduous fruit trees such as plums, peaches, apricots and pears. In no way were they connected with the growing of cruciferous crops. All of the samples were taken in the field and immediately placed in sterile beef bouillon broth tubes. These were incubated and plated. Transfers were made of the resulting colonies.

One hundred and eighty-seven soil samples were secured and cultured. *B. maculicolum* was isolated from 28 of these.

NATURAL OCCURRENCE ON INSECTS

The red-bordered stink or plant bug, *Euryopthalmus convivus,* is quite common in the above deciduous orchards, but it appears to do no particular

harm. It inhabits these orchards the year round. This insect is particularly active and is found crawling about in the trees and on the soil and in the soil cover. The writer was curious to find what relation this insect had to the dissemination of bacteria which cause the gummosis of stone fruit trees. Aphids are also found commonly on these trees, and these were also examined. Some 65 insects were cultured by placing them in beef broth. Some of the insects were treated with alcohol before placing them in the culture fluid; some were treated with alcohol and then crushed in the culture fluid. No organisms were isolated from these last two sources. Sixty-five isolations were made from the remainder and, of these, ten proved to be *B. maculicolum*, showing that the insect carries the organism on its extremities. No organisms were isolated from the aphids.

SUMMARY

1. The economic importance of the spot disease of cauliflower appears to be increasing in the San Francisco Bay region of California.

2. Sharp frosts, especially during the latter part of the growing season, cause an injury which allows a greater amount of infection.

3. The practice of allowing the heads to become over-mature appears to favor the infection.

4. The cultural characteristics and biological activities of the bacterial organism isolated by the writer agree with those described by McCulloch.

5. Young cauliflower plants failed to become infected with *B. maculicolum* when sprayed with recently isolated strains.

6. Cauliflower heads were readily infected when the organism was sprayed upon the fleshy part.

7. *B. maculicolum* readily incites the formation of agglutinins in the serum of rabbits when injected intravenously. A titre of 1/10,000 was secured.

8. The bacterium was able to persist in flasked dry soil for at least a year.

9. The bacterium was found naturally in soils which were not cropped to cruciferous plants.

10. The red bordered stink bug, *Euryopthalmus convivus*, carries the organism on its extremities and thereby acts as a disseminating agent.

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COLD CHLOROSIS OF SUGAR CANE[1]

JAMES A. FARIS

During the winter of 1924–1925 the writer observed peculiar white bands upon the leaves of Yellow Caledonia cane on the south coast of Habana Province, Cuba. Several fields were thus affected, and every plant seemed to be uniformly marked. These markings were very definite bands across the leaves, in which the chlorophyll had failed to develop. No such markings were noticed in the Cristalina cane growing alongside these fields. The country people attributed these white bands, or "manchas blancas," as they call them, to freezing during a period of cold weather, but this explanation seemed out of the question, as no freezing temperatures have ever been recorded from this locality. These fields were again observed in May, 1925, two months later, and no new markings had appeared, but the areas on the older leaves still persisted. No further evidence of such chlorosis was seen until December, 1925, when bands appeared simultaneously upon several cane varieties in the collections of the Cuba Sugar Club Experiment Station of the Tropical Plant Research Foundation, at Central Baraguá, Cuba. In January of the present year specimens affected with this form of chlorosis were sent to the station from Central Carmita by Mr. J. T. Crawley. Other cases have been observed by the writer in Santa Clara, Habana, and Oriente provinces during the months of January, February, and March. In some cases these have been confused with mosaic by the field men. A study of the contributing causes was then made and experiments were begun in which these white bands were produced under controlled conditions, as hereinafter recorded.

DESCRIPTION OF THE CHLOROTIC BANDS

The bands observed range from 2 to 4 inches in length and vary in width from a narrow strip along the margin of the leaf to bands which completely cross the leaf and have every appearance of being formed in the cornucopia of the leaves. All of the central leaves are thus marked, but in later growth the affected areas separate and hence are quite differently located upon the mature leaves. As will be seen from figures 1 and 2, the whitened bands are relatively lower upon the older leaves and are progressively higher upon the younger ones. The youngest leaf affected usually has the band at, or very near, the tip.

While the bands appear to be very regular in their limits, it will be seen upon close inspection that the borders of the white and green tissues are very

[1] Scientific Contributions No. 3, Tropical Plant Research Foundation.

irregular (Fig. 3). A part of this irregularity is due to cell divisions subsequent to the original exposure. The tissues involved in this band seem to have lost the power to form chlorophyll. The cases where distinct green stripes develop along the veins are interpreted as being due to the normal development of chlorophyll in the deeper lying tissues which escaped the cold injury, especially the chlorophyll-bearing bundle sheath which forms a continuation of the sclerenchymatous tissues around the fibrous strands of the leaf sheath and cane stalk. These thick-walled cells continue to develop chlorophyll even after this power is lost by the surrounding parenchyma.

FIG. 1.—Plant of H–109 cane with chlorotic bands due to cold injury.

Since every indication pointed to the formation of these chlorotic bands while the young tissues of the various leaves were in the cornucopia, experiments were devised to test the theory by keeping melting ice above this point and permitting the "boot" of the plant to stand full of cold water. A funnel of tough paper was tied about the top leaves of 27 different stalks of H-109 and D-1135 varieties of cane and was kept full of ice during a period of three consecutive nights. The ice was applied during the nights of Janu-

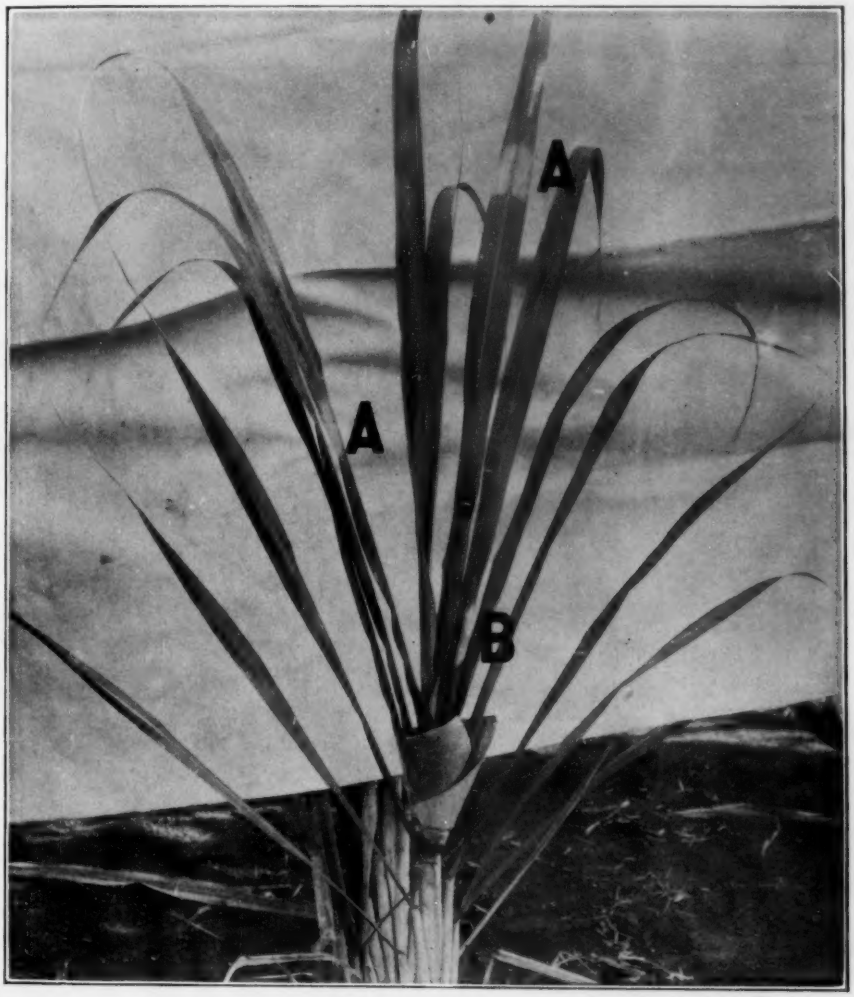

FIG. 2.—Plant of D-1135 cane with (A) chlorotic bands induced by melting ice in the "boot," and (B) induced by cold weather.

ary 22, 23, and 24, 1926. White bands began to develop January 30 upon the treated plants, but no signs of chlorosis developed upon the untreated stalks of the same plants. The bands upon the treated plants were not so marked as bands which developed within a few days after periods of cold weather occurring in December, 1925, and in January and February, 1926. However, artificially produced bands were well marked and definite, as can be seen from the bands indicated as A in figure 2.

A study of the temperatures and rainfall during the season of 1925–26 shows that an outbreak of this leaf banding has occurred in every case where

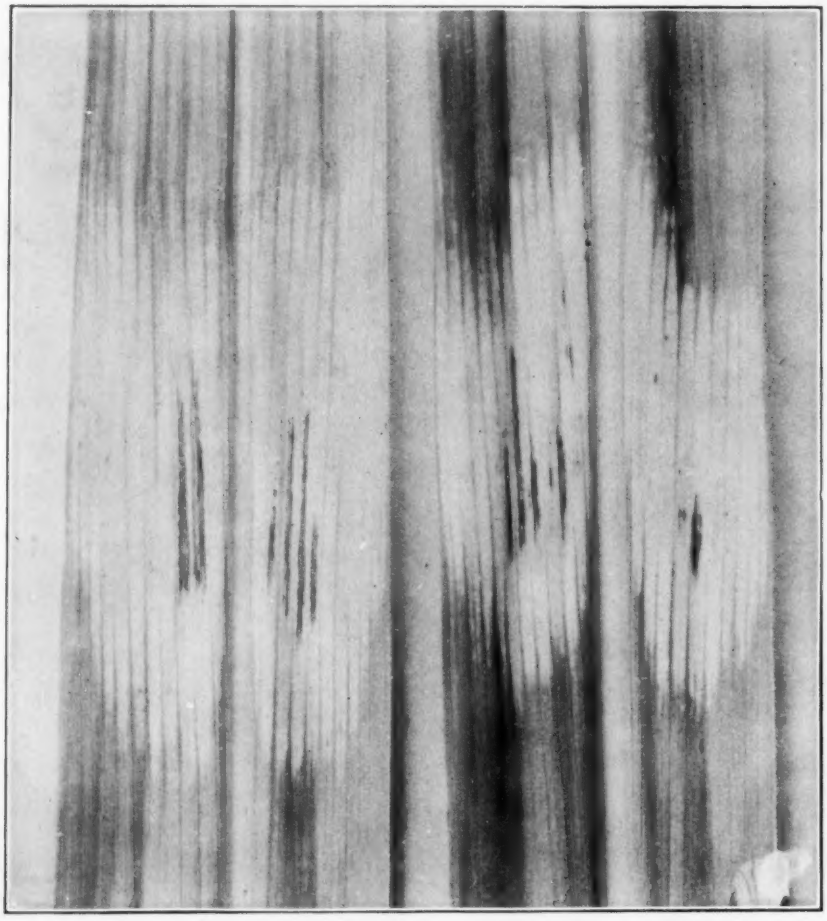

FIG. 3.—Cane leaves with chlorotic areas due to cold injury. The darkened stripes in the chlorotic area are due to the attacks of a weak fungus parasite. Natural size.

low temperatures have followed periods of rainfall. Usually winter rains in central Cuba follow the continental storms of the North and are, as a rule, followed by colder weather. Such conditions stimulate growth in the cane, and then the cold water standing in the cornucopia of the stalk chills the newly exposed tender tissues, thus causing the chlorosis in the leaves affected. This combination of conditions is shown graphically in figure 4, a chart showing the maximum and minimum temperature curves and rainfall at Baraguá from November 1, 1925, to March 7, 1926. The arrows indicate the date of first appearance of the white bands in the leaves of H-109 and D-1135 cane varieties. The chart also shows the dates of application

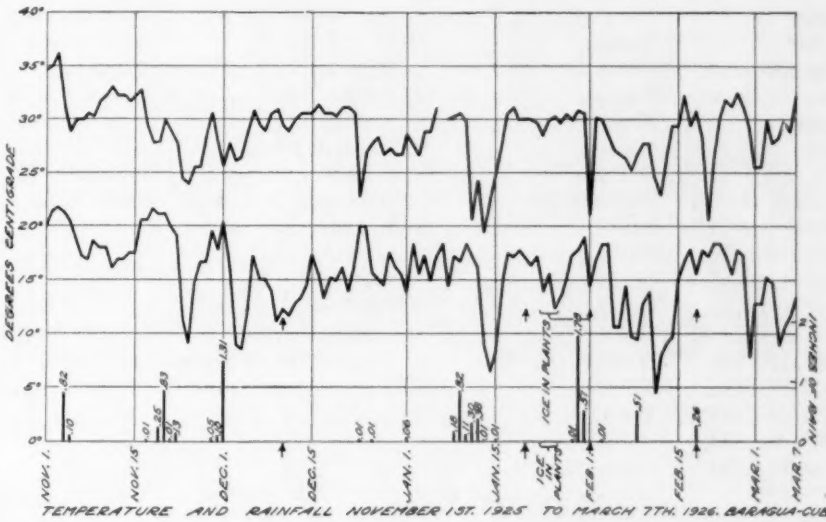

FIG. 4. Chart showing maximum and minimum temperatures and rainfall at Central Baraguá, Cuba, from November 1, 1925, to March 7, 1926. The arrows indicate the dates of appearance of cold chlorosis.

of the ice in the experiments and the appearance of the chlorotic bands thereafter.

From the chart it would seem that two or three nights of temperatures from 5° to 10° C. are required to produce this response in the susceptible varieties.

RELATIVE SENSITIVENESS OF CANE VARIETIES TO COLD CHLOROSIS

Some varieties are much more sensitive to this form of cold injury than others. This does not seem to be so much a matter of the age of the plants as the age of the tissues exposed and their inherent sensitiveness to cold.

Cold weather not preceded by rainfall to stimulate renewed growth may cause the appearance of slight chlorotic bands, while temperatures not quite so low but preceded by weather favorable for growth are followed by very marked chlorotic bands across the leaves. At the temperatures recorded in figure 4 the following varieties (Table 1) developed this form of white bands. The sensitiveness of the varieties is roughly indicated on the scale of 10, using 10 for the most sensitive and 1 for those varieties which developed slight chlorotic edges on the leaves.

Under the same general field conditions at Baraguá, Cuba, the following varieties did not develop these chlorotic bands in the leaves:

Badila
Cristalina
Striped Bourbon
Uba
Oshima
Burra
S.C.–12/4
Tucuman 439
B–3696
Cuba 1–34,
36–37, 40–45,
47, 49, 50, 52,
53, 64, 69, 145,
251, 291, 338,
350, 351, 362,
364, 384, 385,
390, 419, 483,
519, 555, 768
C.H.–64/21
Kavangire
Penang
Criolla
Toledo
Kinar
Banes 2
B–1753
Tucuman 507
B–6450
D–109
D–433
D–247
D–604
D–625
EK–28
M–36
B–208
Gomez Mena 4–6
do 8 and 9
La. Purple
Bocoya
Tekcha
Kassoer
Brava de Perico
B–1809
B–3412
B.H.–10/12
CO–210, 213,
214, 232, 281
P.O.J.–36, 213, 228,
234, 826, 979,
2379, 2714, 2221
Java Unknown
Caña Blanca
Tjepering
Bamboo Blanca
do Morada
B–3405
S.P.I.–33243
B–4596

Some of these varieties might develop this chlorosis at colder temperatures, or even at a greater prolongation of the temperatures recorded in the chart.

LEAF SPLITTING BY SECONDARY FUNGUS PARASITE

As shown in figure 3, when the leaves with these bands reach maturity, the tissues between the veins in some plants of certain varieties are attacked by a fungus which forms an elongated stripe. This stripe has a narrow reddish border and a gray center in which fruits of a Coniothyrium species are often formed. The leaves may split along these stripes, but the midrib and veins are usually strong enough to give mechanical support. The stripes occur on a very small proportion of the affected areas even in the susceptible varieties. They resemble very much the stripes formed upon chlorotic leaves of cane under injurious soil conditions such as areas of coco out-crops.

TABLE 1.—*Relative sensitiveness of cane varieties to cold chlorosis*

Variety	Sensitiveness to cold chlorosis	Variety	Sensitiveness to cold chlorosis	Variety	Sensitiveness to cold chlorosis
H–109	10	Kavangerie	3	B.H. 10/12	1
D–1135	9	Cuba 35	3	C–100	1
D–99	8	Cuba 483	2	D–116	1
D–74	5	P.R.–209	2	D–216	1
C–46	5	P.R.–707	2	F.C.–37	1
Yellow Caledonia	4	B–109	1	F.C.–306	1
		Cayana 10	1	P.O.J.–2725	1

SECTIONAL CHLOROSIS IN HAWAII

At the Honolulu meeting of the International Society of Sugar Cane Technologists,[2] the matter of sectional chlorosis of sugar cane was discussed. Its presence was reported in Australia by Mr. North, and in Hawaii by Mr. Lee. A similar trouble in Louisiana was reported by Mr. Brandes.

Mr. North suggested that the injury was associated with cold weather, as is the case with the cold chlorosis occurring in the fields in Cuba (Fig. 4). This is also confirmed by my experiments with ice in the spindle of the young cane plants. It is not necessary that the temperature should drop to freezing. In the more sensitive varieties temperatures around 10° C. seem to be sufficient to cause the injury. In Hawaii it has been noted that H–109 and D–1135 are very sensitive to the sectional chlorosis. This has also proved to be the case in the cold chlorosis herein reported (Table 1).

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[2] Digest of the Proceedings of the 1st Conference of the International Society of Sugar Cane Technologists, Honolulu, 1924.

PHYTOPATHOLOGICAL NOTES

Virus mixtures that may not be detected in young tobacco plants.—For over a year the writer has used a virus of tobacco mosaic[1] which appears to be free from any other mosaic virus when observations are made on young plants grown at temperatures near 25° C. However, from three to six weeks after inoculations, small, inconspicuous yellow spots (1–5 mm. in diameter) begin to develop, usually on the older leaves. Rarely have more than three or four such spots been observed on a plant. At high temperatures (90°–100° C.) these yellow spots have been observed on 4 out of 400 young mosaic affected plants.

When young tobacco plants were inoculated with virus from these yellow spots and grown at 25° C., yellow mosaic developed in all the plants in from 10 to 12 days after inoculation. Later, green mosaic developed on the younger leaves, and this predominated over the yellow mosaic on the subsequent foliage. However, rather large, yellow spots eventually developed on the larger leaves of the infected plants.

When young plants were inoculated with virus from the green tissue bordering the yellow spots, a slight amount of yellow mosaic occurred in from 10 to 12 days, and later, green mosaic developed also; but when virus was taken from points on the same leaf more remote from the yellow spots, only green mosaic occurred on the young plants.

There are two points of outstanding interest in these results, (a) the suppression of yellow mosaic in the great majority of young plants when this virus is very dilute, and (b) the localized concentration of the yellow mosaic virus in the yellow areas.

These results make it apparent that a virus contamination may not be detected when the observations are confined to young plants. It seems evident that observations should be made for a considerable period after the occurrence of green mosaic in order to detect any possible outstanding variations in the symptoms. It now appears that the virus of the yellow mosaic has been purified by successive sub-transfers from the yellow spots.—H. H. McKinney, Pathologist, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.

[1] This virus was obtained from a single plant which was kindly supplied the writer by Drs. James Johnson and M. Mulvania. The mosaic on this plant was of the typical green type which various workers have described.

Progress report on bunchy-top of abaca or Manila hemp.—Abaca or Manila hemp (*Musa textilis*) is one of the leading export crops of the Philippine Islands. Its most serious malady is bunchy-top, which is considered to be a virus disease. In all probability this is an old disease of abaca. The writer noted symptoms of it in the province of Albay, Island of Luzon, in 1910 and 1911. Dr. Otto A. Reinking brought a specimen from the same island from Silang, Cavite Province, on April 7, 1925. The alarming proportions of the disease in the abaca regions of the Philippines has recently attracted the attention of growers, and the writer's interest in bunchy-top was stimulated by a conference with Dr. Reinking.

The most striking characteristic of bunchy-top is the crowding of the leaves into a more or less rosette arrangement. Infected plants are much stunted and produce short pseudo-stems. Observations indicate that infected plants never recover. They die prematurely, and very few of them, if any, produce fruits. Abaca is susceptible to the disease at all times during its life cycle. Aphids were noted on bunchy-top infected abaca in Los Banos as early as June, 1925. On October 23, 1925, they were found in abundance on the unexpanded youngest leaves of healthy and bunchy-topped plants. This aphid has been identified as *Pentalonia nigronervosa* Coq. by Doctor L. B. Uichanco. It is of interest to note that in Queensland, Australia, bunchy-top of banana is reported to be transmitted from plant to plant by the same species.[1] We are led to believe that this aphid plays an important role in the transmission of bunchy-top of abaca in the field.

Results obtained from experiments using *Pentalonia nigronervosa* as vector indicate that bunchy-top may be transmitted from diseased abaca plants to healthy ones by this aphid. Material from several sources was used in the preliminary experiments, namely, young abaca plants grown in the Plant Pathology experimental plots, infected plants in the Agronomy field, and plants grown from seeds. Since bunchy-top plants do not produce fruits, disease-free plants were obtained from seed. Seeds were germinated in flats, and after emergence the seedlings were transplanted to pots where they were screened by cheesecloth. These seedlings were used in transmission experiments. Further work is in progress.—GERARDO OFFIMARIA OCFEMIA, Department of Plant Pathology, College of Agriculture, Los Banos, Philippine Islands.

[1] Anonymous. Bunchy top in bananas. Cause and nature of disease. Report of Investigation Committee. Queensland Agric. Jour. **24**: 424–429. 1925.

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